

Hedging & fence-sitting from the Think Tank

ONE of the prime skills of the politician is to find the right words to ensure that nothing is given away during debate. The academic politician, canniest of them all, is usually able to find the most eloquent way of keeping open all options, and even in response to the most mundane question will unearth a convoluted answer. "Will all the delegates be coming in to dinner?" the Master of a Cambridge college was asked recently. "Some . . . but perhaps by no means all" was his reply. The Think Tank's latest offering, *Energy Conservation* (HMSO, £1.00) is replete with such generalised High Table fence-sitting.

The Central Policy Review Staff (CPRS) set itself—at least nowhere is there any indication that the idea sprang from elsewhere—the task of producing a document for public discussion on how energy conservation might be achieved. A broad interpretation was put on the words 'energy conservation' and the CPRS included an analysis of methods of using replenishable forms of energy as a means of conserving fossil-fuel-generated energy. We reported last week the outlines of the report. What concerns us here is whether the CPRS does a good job when venturing into such fields and whether energy conservation is an appropriate matter for the CPRS to study in any case.

The most striking feature of the report to a scientist is the utter lack of any substantiating material for all that is said. In the report's 64 pages it roams widely over material as varied as domestic insulation and energy from sea waves. The authors (anonymous, of course) have clearly done a lot of reading and presumably have also been to see many of the scientists and technologists whose work forms the basis of the report's conclusions. Yet nowhere is there a single reference to published work, nor to a conversation with anyone. As a result it is extraordinarily difficult for this document to be a vehicle for public discussion. Beautifully manicured paragraphs that would have delighted Gowers for their style and clarity are no substitute for well documented arguments.

Consider, for instance, the Severn barrage scheme. It is described and dismissed in two paragraphs. "Significant environmental benefits are claimed by proponents [here follows a list of benefits] . . . They are not, however, easy to substantiate and would not appear to transform an apparently uneconomic project into an economic one . . . The long term ecological effects are very uncertain." This may be an elegant way of covering a lot of ground but it leaves the reader with too many unanswered questions—who are the proponents, why are the benefits not easy to substantiate, who is concerned with long term ecology? Or again in discussing energy storage schemes, " . . . nevertheless, the widening

differential between nuclear and fossil fuel costs in favour of the former may prove sufficient to make at least some of these schemes economic." Some, but perhaps by no means all.

The central question, however, is whether the CPRS should be doing this sort of study at all. There are various spurious arguments that can be raised against its involvement, such as that the staff cannot possibly be technically qualified or that the subject is more properly a departmental matter. If a Think Tank is a good thing, and we believe it to be so, then it must certainly be allowed to do what it wants and to risk offending the specialist departments. What it must not do, for its own sake, let alone that of taxpayers, is to bite off more than it can chew. This it has palpably done in this case.

Not only is the subject of energy conservation colossal in extent, but the CPRS has expanded it even more by discussing the economy of the inexhaustible resources. With such a wide field it is almost inevitable that there will be no incisive thinking, since the staff, desiring to be comprehensive, can have had little time to develop expertise in individual areas. The overall impression that the report gives is of diligent reading—and there has been ample material to read in the last year—but no very profound thinking. The document bears no marks of being the subject of intensive discussions amongst the staff; indeed it seems instead the sort of thing that an academic might produce during a sabbatical year. This, surely, is not what the Think Tank should be all about. If, in the future, the CPRS does not want to get a reputation amongst scientists for vapid thinking it will have to concentrate its attention much more.

100 years ago



If anyone wants to see how lamentable is the absence of practical work in the examination system of the University of London, let him get "Questions in Chemistry and Natural Philosophy given at the Matriculation Examination of the University of London from the year 1864 to June 1873, classified according to the syllabus of subjects," by C. J. Woodward, B.Sc. (Simpkin, Marshall, & Co.) We say nothing against the book itself, which is a creditable compilation of its kind, but the system capable of giving birth to such a text-book must be an unmitigated encouragement to "Cram."

From *Nature*, 10, 215, July 16, 1874.



Gamma-ray astronomy: the last observational frontier

ON June 10, 11 and 12 a symposium on "The Context and Status of Gamma Ray Astronomy" was held at the ESRIN laboratory in Frascati. The rationale behind such a symposium under the ESRO banner at the present time derives partially from the forthcoming launch of COS-B (see *Nature*, **249**, 398; 1974); but quite apart from this slightly parochial incentive it turned out that gamma-ray astronomy has indeed reached something of a landmark recently, and is now likely to fulfil some of the promise which it has held for so long.

Certainly throughout the 1970s we have heard repeatedly that the big breakthrough in gamma-ray astronomy 'is about to be made'. But the difficulties of first detecting any gamma-rays from outside the Solar System and secondly deciding from exactly which direction they are coming have made some of the premature claims ring rather hollow in recent years. One result of these difficulties has been a considerable broadening of the accepted definition of gamma rays in this context; some of the data presented at Frascati would have been equally well suited to a symposium about X-ray astronomy. Such definitions are, however, intrinsically arbitrary, and as long as the active observers know what they mean by gamma rays no harm is likely to result from a broadening of the definition.

Ironically, in spite of the intensive efforts made to detect gamma ray events from outside the Solar System the most impressive data gathered so far have come from a series of satellites designed to monitor events on Earth. These, of course, are the Vela satellites, which are intended to detect radiation from nuclear explosions on Earth. Over the past four years, these satellites have not had the opportunity to discover many terrestrial nuclear explosions; but the same laws of physics apply to nuclear explosions elsewhere in space, where bursts of gamma rays are produced. As a result, the various Vela satellites have marked all of the 30 or so gamma ray bursts which have been recorded so far. And in only a couple of cases was it necessary to

A recent symposium provided an insight not only into the present status of gamma-ray astronomy, but also into the conventions of scientific symposia, as John Gribbin reports.

examine the Vela data in the light of evidence gained from other satellites before the evidence of the bursts became clear.

So gamma-ray astronomy can definitely be said to have cleared the first hurdle, of detecting something definite. The second problem has also been at least partially resolved since the proliferation of satellites equipped to detect gamma-ray bursts has made direction finding, by a kind of astronomical triangulation, a feasible proposition. And this, more or less, is where the Frascati symposium comes in. Other topics were also discussed, covering the gamma-ray background, low energy gamma-ray astronomy, and galactic emission. But to an observer from outside the gamma-ray astronomy family, the bursts and their interpretation provided the dominant interest.

I. B. Strong (Los Alamos) reviewed the observational data concerning the bursts, and other contributors elaborated on specific measurements before F. Pacini (Frascati) reviewed theories relating to their origin. In both branches of the investigation, the pioneering nature of gamma-ray astronomy was clearly apparent—but the effect of this on experimentalists and theoreticians differed greatly.

In a very real sense, gamma-ray astronomy is the last frontier of observational astronomy. It is now possible to observe every part of the electromagnetic spectrum, and the gamma-ray region is the last for which practical techniques have been developed. The observers, many of whom have a background in particle physics rather than in astronomy, seem somewhat overawed by this, and perhaps by the long struggle they have had to get hold of any worthwhile data. The result is that each observation seems to

be exhaustively analysed and picked over for significance—and the danger, of course, is that too much might thereby be read into what may well be atypical, or even incorrect, data.

The theoreticians, however, have fewer inhibitions. Indeed, a great many theoretical astronomers delight in a situation where there is just enough evidence to make model building worthwhile, but not enough to prove that their favoured model is incorrect. The study of gamma-ray bursts today provides a happy hunting ground for such theoreticians.

The fondness of the observers for smothering detail extended even to the presentation of their contributions at the conference. In the most extreme cases, we were told openly that although only a short paper was being presented, the version in the published proceedings of the conference would be much more 'complete'. That hardly seems fair on participants (some of whom had come from the United States and Japan), let alone on the humble reporter. In addition, many of the observations seemed essentially to duplicate one another, so that we had a stream of speakers standing up and saying much the same things about various events observed by different satellites, and showing graphs which seemed to be essentially interchangeable, allowing for the largish error bars.

Is this kind of presentation really necessary? Clearly, each group must present its results and have them published in the proceedings of such a key conference, or the holders of the purse strings will want to know what is going on. But if each group had provided a summary of its data, circulated to participants in advance, and one or two speakers had outlined the implications then I feel sure that the symposium would have been just as fruitful, and perhaps a lot less tedious in parts.

Much the same could be said about most scientific symposia. But in the case of gamma-ray bursts such a streamlining of the proceedings would have been particularly apt, since only about three things are known about the bursts. First, they occur about seven times a year. Second, they are

very sharply defined in time with perhaps a double structure. And third, on the basis of 12 direction measurements, their distribution seems isotropic.

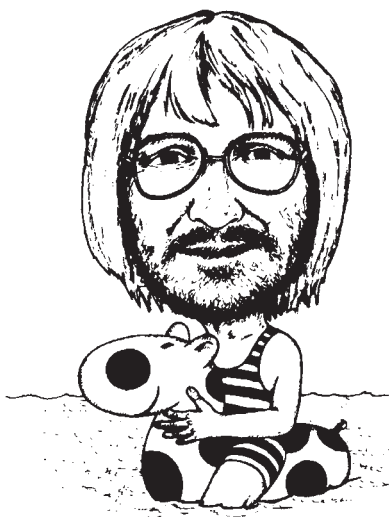
Of course, much more in the way of detailed measurements is available, and was presented. But that is just about all the evidence on which the theoreticians can build their models. Nothing daunted, the theoreticians have proceeded to do just that, as Pacini described.

The isotropy of the burst distribution provides vital information. Either the bursts originate nearby (a few hundred parsecs from the Sun), or they must be extragalactic. The intermediate case, of a distribution over a large part of our Galaxy, would reveal structure related to the structure of the Galaxy. With that proviso, Pacini happily listed the models which have been proposed: radiation from relativistic dust grains entering the Solar System; comets falling into collapsed stars; traces of "defunct" pulsars; superflares on stars; supernovae in external galaxies; birth of neutron stars; and the "first manifestation" of radio outbursts in external galaxies.

It does not really matter which, if any, of these models may turn out to be correct. What is significant is that theoreticians are prepared to toss such ideas into the melting pot, throwing them out or revising them as observations require. The observers, on the other hand, seem to wish to build complete detailed models on the basis of incomplete data, while justifying the procedure with a barrage of statistics (not always valid for small samples) and the interchangeable, large-error graphs which I have already mentioned.

Perhaps this is a result of the lack, in some cases, of astronomical training. With experiments which simply cannot be repeated and the everyday hazards of balloon and rocket observations, something more of the pioneering attitude seems to be needed in this last frontier of observational astronomy.

But on the other hand, this earnestness among the observers is really only manifested when they get up on their hind legs to address a gathering of



John Gribbin (left) lives by the sea at Brighton.

Glaxo awards

JOHN Gribbin, of *Nature*, is among the four recipients of Glaxo Travelling Fellowships for science journalists announced on July 9. His award, in the National category, is chiefly for a series of articles on the significance of climatic change; much of this work has appeared in *The Times Science Report* and longer articles appeared in *Nature*, *New Scientist* and *Environment and Change* during 1973.

The awards, which are sponsored by Glaxo and administered in collaboration with the Association of British Science Writers, are in the form of £500 travel grants; John Gribbin will be using his award to travel to the United States and Canada during the autumn, and will be reporting for *Nature* on research into climatic

change being carried out there.

John Gribbin joined the staff of *Nature* in October 1970, after completing research for a PhD in astrophysics at the University of Cambridge. In that year, he received the First Award of the Gravity Research Foundation of New Boston for work on "Using Gravity to Determine the Nature of Superluminous Astronomical Objects"; for most of the past four years he has been in charge of the *Nature-Times News Service*, which reports on developments in all the sciences. As well as the interest in climatic change which has led to this award, John Gribbin is concerned about the application of science to other global problems, and is co-author of a book on earthquakes and earthquake prediction (*The Jupiter Effect*) which is to be published by Macmillan in September.

Fellowships in the other three categories (Radio and Television; Regional; and Trade, Technical and House Magazine) go to David Wilson (Science Correspondent of BBC Television News), Judith Hann, a freelance science journalist, and Geoffrey Watts, Deputy Editor of *World Medicine*. Miss Hann is the first person to win a Glaxo Fellowship for the second time; the first occasion was in 1967.

Last year's award winners included John Maddox, then Editor of *Nature*, in the National category.

their peers. Individually or in small groups (especially after lubrication with the local Frascati wine) they appeared as intelligent people with the sense of humour needed to cope with the rigours of their trade. In some cases, they even agreed that their data were really rather vague and open to other interpretations, at least in detail.

The symptoms are, indeed, very similar to those severe cases of jargonese which result when many people try to write a "scientific paper". In the long term, the answer to both problems must lie in a basic change of attitude in the direction of clarity and honesty of communication as opposed to cunning packaging which can only be interpreted by the initiated.

In the short term, since almost every scientist is willing to communicate as a human being on a face to face basis, saving the 'scientist' mask for lecturing,

a solution might be to cut down on formal presentations at symposia, in favour of circulation of papers together with ample opportunity for informal discussion. At Frascati, more than 50 talks were on the official timetable (covering 2½ working days) and more were crammed in at short notice. Apart from the obvious benefits of a trip to Italy in June, it is difficult to see how the participants would have been less well served simply by reading the papers. The advantages of a symposium should be personal contact and the opportunity to assess the abilities of colleagues in the same field who may work on another continent; it seems that the time has certainly come when a move away from the present system, in the direction of the original "drinking party" implications of the word "symposium" could well be a good thing.

international news

EVER since the United States Supreme Court ruled last year that abortion is essentially a private matter between a woman and her doctor, a coalition of organisations campaigning under the banner of the 'Right of Life' movement has been trying to make it again a criminal matter between a woman, her doctor and the courts. The prime objective of the campaign is to get Congress to pass an amendment to the constitution to "protect the life of the unborn", but the chief casualty so far, much to the consternation of many biomedical scientists, is research of human foetuses.

The list of restrictions which have been placed on such research is impressive. Congress last month passed a bill which imposes a four-month ban on research on living foetuses while a special commission will draw up ground rules under which foetal research should be funded. The National Institutes of Health (NIH), which claims that it does not support research on live, aborted foetuses anyway, is drafting regulations governing all research of human subjects, including foetuses. An amendment has been put into the authorisation bill for the National Science Foundation (NSF) forbidding that agency to support research on live foetuses. (Backers of the amendment were evidently unmoved by protestations that NSF has never been in the business of funding foetal research, and does not intend to be.) On June 26, a bill was signed into law in Massachusetts which makes it a criminal act for anybody to carry out research on living foetuses there. And finally, a law has been on the books in Illinois for a year now which forbids research on all "aborted tissue" in that state.

Aside from the Illinois law, which imposes a blanket ban on all foetal research, the restrictions and prohibitions apply chiefly to two areas of study. First, they rule out research on foetuses aborted during mid-pregnancy which are delivered with their hearts still beating but which are insufficiently developed to remain alive for more than a few seconds or minutes. And second, they ban most research projects on foetuses while they are still in the womb. It is the latter area which is at the centre of most of the disputes, for research on foetuses *in vivo* has already produced some shortsighted and ill-founded conclusions.

At the federal level, the issue of

Foetal research aborted in United States

by Colin Norman, Washington

foetal research burst into the public spotlight last year following newspaper reports that NIH was drawing up guidelines for research on living foetuses. The day after the report was published, Dr Robert Berliner, then deputy NIH director for science, told about 200 Catholic high school students who had descended on the NIH campus in protest that "we know of no circumstances at present or in the foreseeable future which would justify NIH support of research on live, aborted human foetuses." He added that no such research was then being supported.

Nevertheless, anti-abortionists in Congress, led by representative Angelo Roncallo, a republican from New York, proposed a series of amendments to various bills which would have ruled out entirely all federal support for research on living foetuses. It was largely to head off such restrictive measures that a provision which imposes a four-month ban on such research was put into a bill dealing with biomedical ethics. The bill, which cleared Congress late last month, simply states that the Secretary of Health, Education and Welfare cannot support research on a living human foetus, "unless such research is done for the purpose of assuring the survival of such foetus". While the ban is in effect, a special Biomedical Ethics Commission will draw up rules governing such research.

As for the Massachusetts law, its implications were neatly summed up last week by Dr Elizabeth D. Hay, Professor of Embryology at Harvard and past president of the Society for Developmental Biology, who pointed out that "the federal ban cuts off funding for foetal research, but the (Massachusetts) state law will put you in prison for up to five years".

Sponsored by William Delahunt, a young state legislator, and vocally backed by the Boston Citizens' Committee for the right to life, the original bill would have ruled out most foetal research in Massachusetts, but it quickly fell foul of the Boston scientific community, which ran an effective

lobbying campaign to make the measure less restrictive. In its final form, the bill prohibits research in Massachusetts on live human foetuses, before or after abortion, but it also contains two important exemptions. First, research will be allowed if it has potential therapeutic value to the mother or the foetus and, second, it does not ban disgnostic tissue from dead foetuses, provided that the mother's consent has been obtained.

Asked for their opinions on the various federal prohibitions and the Massachusetts law last week, a number of biomedical scientists offered two chief observations. The first is that foetal research has provided many very significant findings, and a good deal of direct benefit to public health. One piece of research which is often cited in that regard is the growing of polio virus in cells cultured from foetal tissue, for which Dr John Enders and Dr Thomas Weller won the Nobel Prize in 1965. The second is that it is inadvisable to legislate an overall ban on any area of scientific research—such matters are best dealt with by ethical review committees which, according to federal regulations, must be established to guide research in institutions which receive biomedical research funds from the government.

What, exactly, is going to be ruled out by the various restrictions? The first thing to be said is that the ban on research on foetuses whose hearts are beating after they have been aborted will have little effect in the United States because there is very little of that kind of research going on there. For one thing, the usual method of abortion during the second trimester is saline injection which usually kills the foetus. Some research on foetal circulation and on the development of the immune system has, however, been carried out by American scientists in Scandinavia, where the preferred method of abortion during later stages of pregnancy is Caesarean section, and the federal ban will clearly rule out all NIH funding of such studies.

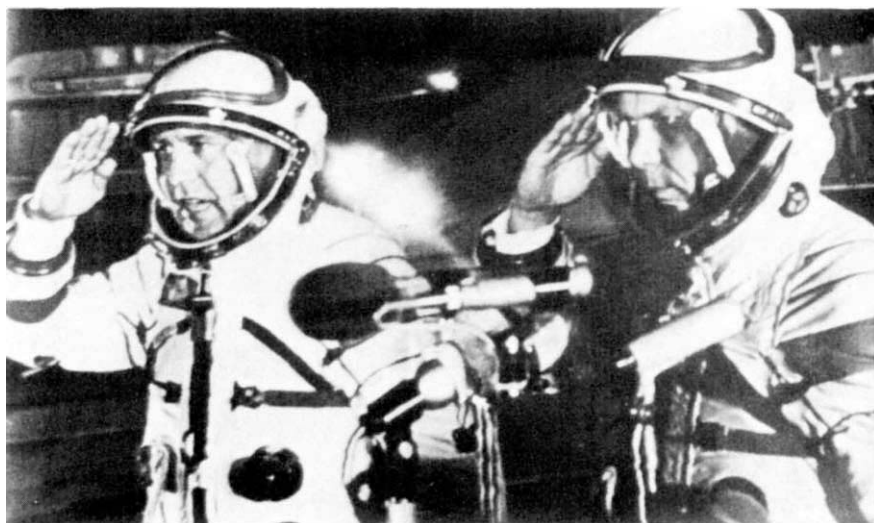
According to Dr David Nathan, a scientist at Boston's Childrens' Hospital, and a leading opponent of the Massachusetts legislation, the chief result of the various prohibitions will be "two serious losses, one in the area of foetal pharmacology, and the other in the screening of teratogens and dangerous drugs", both of which will be ruled out by the bans on research

THE launching of the space station Salyut 3 on June 24, 1974, and the successful docking with it of Soyuz 14 and the transfer of the two-man crew on July 5, marks a resumption of the Soviet programme of manned orbital space stations which was tragically interrupted in June, 1971 with the death of the three-man crew of Salyut 1 during re-entry.

In the three year moratorium, modifications have been made both to the Salyut station itself, and to the Soyuz programme which supports it. The re-designed Salyut was officially flight-tested (unmanned) during April 1973. This craft incorporated modifications to the solar panels (three panels rotatable through 180°, instead of the previous four) and to the optical ground-viewing systems. In addition to this "official" test (Salyut 2), other craft of the series have almost certainly been tested, unnamed, under the cover-all of the Kosmos programme.

The Soyuz, too, was almost certainly tested out as a number of Kosmos satellites (numbers 496, 573, 613, in particular, are widely considered to have been unmanned Soyuz craft) before manned testing was resumed with Soyuz 12 (launched September 29, 1973—a two-day flight) and Soyuz 13 (launched December 18, 1973—an eight-day flight).

The principal modification to the resumed Soyuz programme was the use of a two-man, rather than a three-man crew. In view of the cause of the Salyut 1 tragedy—the failure of a seal and loss



Cosmonauts Popovich and Artyukhin reporting ready for take-off on July 4th.

of cabin pressure during re-entry, this is almost certainly intended as an additional safety precaution, providing the additional space needed for the crew to wear spacesuits during re-entry. This policy is continued with the latest flight, and it is significant that the *Pravda* picture of cosmonauts Pavel Popovich and Yuri Artyukhin, apparently inside the Soyuz cabin, shows them in space suits.

The official aims of the test according to the Tass reports are: "Further elaboration of the improved construction of the station and also of the on-board systems and apparatus, and the conducting of scientific and technological investigations and experiments in cosmic flight". This includes observa-

tions of geomorphological features of the Earth's surface and atmospheric phenomena, investigation of the physical parameters of space, and medical and biological studies of the effect of "the factors of space flight on the human organism and the 'determination of rational regimes of the crews working' on board.

This last aim seems to be of particular significance in view of the joint Apollo-Soyuz mission planned for next year. Although the official aims make no specific mention of this, the timing of the flight, to coincide with President Nixon's visit, is surely intended to imply that the Soviet preparations for the joint United States-USSR mission are well in hand.

on fetuses before induced abortion.

Specifically prohibited will be research projects which, for example, involve the administration of drugs or vaccines to a pregnant woman scheduled to undergo an abortion, in order to see whether they cross the placental barrier and affect the foetus. Such studies have, however, produced some extremely important findings.

A study carried out in Helsinki and reported in 1972, for example (Vaheri *et al.*, *New Engl. J. Med.*, **286**, 1071; 1972) showed that virus from rubella vaccine can cross the placenta and enter the foetus. The research, which consisted of vaccinating ten women before they had abortions and analysing the foetal tissue for signs of rubella virus, was described by a Harvard scientist last week as "fantastically important", since it provides very strong evidence against vaccinating women during early stages of pregnancy. The research was particularly significant since previous studies had indicated that the virus from the rubella vaccine does not cross to the

foetus through the placenta.

It is also worth pointing out that four doctors are now awaiting trial on criminal charges arising from a research project they conducted at Boston City Hospital in 1971 and 1972. The study consisted of administering antibiotics to 33 women about to undergo abortions to see which was the most effective in crossing the placenta. The idea was to find the best drug to use in place of penicillin to clear up foetal infections. But the four doctors were charged earlier this year with infringing an obscure 19th century Massachusetts law which was originally designed to prevent grave robbing. Although Delahunt said last week that his bill was in no way connected with those indictments, it is clear that if the grave robbing law is found ineffective in preventing such research projects the new Massachusetts law will certainly do the job.

Two chief arguments have been raised against such research, for one thing, it puts the mother who has consented to take part in the study in a

position in which it would be difficult to change her mind about having an abortion. And for another, it constitutes research on a human being (the foetus) without its consent, which is contrary to every code of biomedical ethics including the Nuremberg Code, which was promulgated in response to Nazi medical experiments. Such a view point rests, however, on the assumption that life begins at conception rather than at any other stage of foetal development—an issue which was deliberately left vague by the Supreme Court when it issued its historic abortion decision last year.

Dr Michael N. Oxman, a virologist at the Children's Hospital Medical Center in Boston, argues, however, that a ban on foetal studies *in vivo* "creates a situation where children are going to be put at risk rather than foetuses which are going to be aborted". He pointed out that if a vaccine or a drug administered during pregnancy crosses the placenta and harms the foetus, it would be better to know about it through research on

What that treaty means

LAST week Mr Nixon and Mr Brezhnev signed a treaty on the Limitation of Underground Nuclear Weapons Tests. It was probably the most substantial arms-control agreement to come from Mr Nixon's visit to Moscow and it bears some scrutiny.

- It is bilateral. There is no provision for any further country to attach itself to the treaty.

- After March 31, 1976 the yield of tests may not exceed 150 kilotons. The wording indicates that the United Kingdom, for instance, would not be allowed to test more than 150 kilotons on United States property.

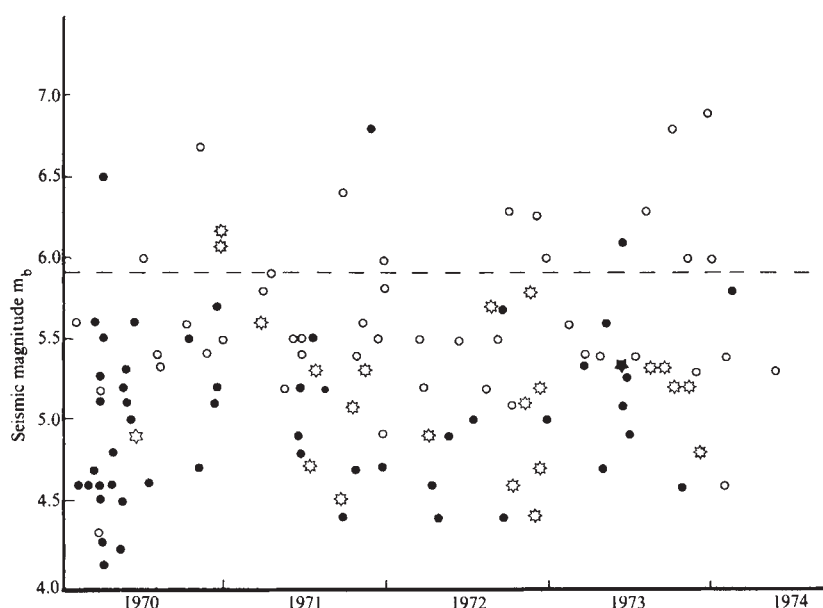
- 'National technical means' will be used for monitoring and neither party will interfere with the other party's means. This leaves much obscure, particularly the question of what could be regarded as interference. If a test of 200 kilotons is fired in an appropriate environment to reduce the apparent yield as seen by a seismometer to 150 kilotons, is this interference?

- Peaceful explosions are excluded from the ban; negotiations on these will continue.

- The treaty must be ratified.

- Data shall be exchanged on the geological and geophysical environment of present test sites and of any future site. A tall order for an area as complicated as southern Nevada.

- Locations of tests shall be exchanged after firing. But the United States, and doubtless the Soviet Union, test many devices much too small to be monitored by national technical means. Are these to be included?



Open symbols—Soviet tests. Filled symbols—U.S. tests. Circles—weapons tests. Stars—peaceful explosions. The dashed line roughly represents a yield of 150 ktons.

- Yields of two tests in each geographically distinct area will be exchanged for calibration purposes.

Reaction amongst those with a long-term interest in a test ban has been bitter. The treaty is blatantly bilateral—there is no provision that any of the data exchanged need get beyond Kremlin and Pentagon files. The extraordinary length of time before the treaty comes into force permits no end of significant tests to be performed at leisure. The threshold is much higher than anyone had believed possible. The figure shows testing

activities for the last four years. It is difficult to relate yield to seismic magnitude in a unique way, because of varying geological environments, but a value of 5.9 might roughly represent 150 kilotons. It is obvious how much testing will still be permissible. But the thing which most people are perturbed about is the way this treaty appears to close out, at least for the immediate future, any thought of a comprehensive ban. The United Nations Disarmament Conference in Geneva will hear some vigorous opinions from the other nuclear powers and the potential proliferators in the next two months.

foetuses about to be aborted rather than through damage to children.

As for the exemptions in the Massachusetts legislation, opinion is divided on how effective they will be in allowing potentially valuable research to take place. Nathan, who last week praised Delahunt's willingness to compromise on the bill, welcomes the provision which allows research on diagnostic techniques likely to be of value to either the mother or the foetus. The exemption will allow research aimed, for example, at developing a foetoscope capable of taking foetal samples for the diagnosis of such diseases as cystic fibrosis and Cooley's anaemia.

But other researchers have pointed out that many valuable techniques and research findings have been developed from research which at the time it was conducted had little potential benefit

for that particular pregnancy. The technique of amniocentesis—removal of fluid from the amniotic cavity—which has led to identification of more than 50 diseases before birth, for example, was used first to measure intrauterine pressure during labour, and would probably never have been developed under the terms of the Massachusetts law. That piece of research would definitely have been ruled out under the terms of the federal ban on foetal research since it was not aimed at "assuring the survival" of the foetus.

Finally, many researchers are concerned that restrictions in foetal research, particularly when they carry possible criminal penalties—the Massachusetts law makes live foetal research a crime punishable by 1–5 years in prison—will drive good scientists away

from important studies. Oxman said, for example that the Massachusetts bill "creates an unfortunate atmosphere in which a good researcher will decide to do something else. The loss could be serious."

Mark V marks time

The University of Manchester has announced "with regret" the decision by the Science Research Council that it will not be able to accommodate the cost of the proposed Mark VA telescope at Meiford, Wales, in its budget "over the next few years". Tenders for the 375-foot instrument indicate a cost of £16–£17 million; the SRC and the University will now have discussions in the near future about more modest possibilities for developing the progress of research at Jodrell Bank.

As a doctor and researcher in medicine and bioelectronics who has always stressed the human mission of medical science and the necessity for international cooperation, I am appealing to you for moral support in my apparently hopeless endeavour to return to active and creative scientific work. For four years now I have been refused all opportunities to work scientifically and professionally in my country and for this reason I applied in 1972 to our Ministry of Health for permission to work abroad. After a year's delay, permission was refused. I have recently sent a new application to the Ministry of the Interior asking for a long term permit to work at research institutes in the United States or West Germany; in the event of this permit being withheld, I have applied to emigrate.

I have been led to this decision by bitter experience with Czechoslovak officialdom and employers, and by the realisation that, as a citizen who in 1968 supported the humanistic reform of socialism undertaken under the leadership of A. Dubcek, I stand no chance in my own country of making any contribution to society according to my skills.

Following the change in leadership of the Czechoslovak Communist Party and the change in party policy, all kinds of repressive measures were, as is generally known, instituted against supporters of the democratic reforms. I suffered harsh reprisals in 1970, when the Minister of Health, without stating any grounds, ordered my immediate dismissal from the post of Director of the Research Institute for Electronics and Modelling in Medicine, the institute which I founded and built up. Then an unprecedented check-up was made in the institute in order to gather evidence against me concerning its economic management. The intentions underlying this action were obvious, and I would be willing to publish the evidence should circumstances demand. At the same time officers of State Security carried out an extensive investigation of many staff members of the institute, and I myself was subjected to repeated interrogation.

Since 1970, after dismissal from the directorship, I have not been allowed to engage in research, experimental or teaching work of any kind, and since 1971 I have even been banned from clinical work. In 1972, on returning from a spell of sick leave, I was not allowed to resume work at my place of employment, although such an order was in contravention of valid Czechoslovak law. I was also banned from publishing anything at all. Scientific papers in the press had to be thrown out, and my name had to be deleted from collective works or was deleted by the censors. Nor can my name

appear in literary references.

By degrees I was expelled from all Czechoslovak professional societies, from membership of the editorial boards of scientific and popular scientific journals, from the Collegium of Medical Sciences, from the Scientific Council of the Ministry of Health and from all technical commissions, and I was banned from lecturing at Prague and Brno universities and at the Prague Institute for Further Education of Doctors and Pharmacists. I am officially excluded from participating not only in conferences abroad but also in medical conferences and meetings at home.

For a full four years I have tried to find a place in society in accordance

**I have been subjected
not merely to social
discrimination but also
to a form of protracted
and total spiritual and
intellectual liquidation
against which I have
no defence**

This is the text of an appeal addressed to the World Federation of Scientific Workers by Bohumil Peleska, a Czech scientist

with my qualifications, I have paid dozens of visits to officials of the Communist Party, to political offices, and to government and public institutions, I have made dozens of proposals as to how I could be employed. All efforts have been in vain, and equally fruitless have been applications to employing organisations; they always cite orders from superior departmental and political bodies. No account was taken even of the fact that, in the course of twenty years of work in this field of research, I built up the Experimental Department of the Institute of Clinical and Experimental Surgery, that I founded and built up the Research Institute for Electronics and Modelling in Medicine, and that I was awarded by the President of the Republic in 1959 the Order of Merit for Services to Construction and in 1965 the Klement Gottwald State Prize for research, nor that I have published 150 scientific works.

The persecution culminated in 1974

when, in contravention of the Labour Code, I was dismissed from my employment under Article 46 on the grounds that I had committed a breach of the social order by my political attitude in 1968. Knowing that colleagues who had suffered dismissal under this Article were in financial straits and searching for jobs, I appealed against the dismissal, but my employers and the trade union refuse to rescind this illegal act, so that now I am not only barred from scientific and professional work—I am unemployed and have no prospects of being able to earn a living for my family, which includes three young children. That is how things are, although the Communist Party and the Czechoslovak government officially maintain that nobody can be made to suffer on account of their political views.

I have been subjected in recent years to a procedure which I consider to be not merely social discrimination but also a form of protracted and total spiritual and intellectual liquidation against which I have no defence at present. The feeling that the rights guaranteed by the Constitution and the Labour Code are inaccessible has convinced me that further efforts to find work in my profession would merely involve further loss of time and of the creative years of my life.

Having, for four years, been banned from scientific and professional activity, from publishing and teaching in the field of medicine and bioelectronics, having been illegally dismissed from my employment and having been repeatedly interrogated by State Security, I have lost the sense of legal and personal security; this has induced me to apply to the Czechoslovak authorities for a long term permit to work abroad, accompanied by my whole family, a step which I prefer to emigration. This demand is in accordance with the spirit of the Charter of Human Rights of the United Nations, on ratifying which the Czechoslovak government also undertook to observe and implement it.

As my previous application was rejected, I am approaching you, not only as an administrative body but also as the conscience of the world community of scientific workers; I ask for moral support in my endeavour to return to scientific life and creative work. Through the widespread and unselfish cooperation among scientists the world over, the fruits of medical research are part of the common cultural wealth of all mankind. Thus no power on Earth has the right to curb, prevent or forbid free scientific cooperation in medicine, or to isolate scientific workers and treat them as serfs merely because they wanted a little more freedom, but not nearly as much as they are entitled to as human beings.

correspondence

Synchrotron radiation facility

SIR,—After reading the article by John Gribbin (*Nature*, April 12) on the proposal for a new synchrotron radiation facility, I am wondering if he and I went to the same meeting. Certainly the meeting I attended did not show clearly that the proposal to build a new £2 million storage ring “needs careful rethinking” or that “the plan as originally envisaged seems unlikely to survive”. At the conclusion of the meeting, the physicists, chemists, biologists and metallurgists present made it overwhelmingly clear that they firmly supported the existing SRC plans.

The project is not at the early stages of planning. Indeed, the scientific case for a dedicated storage ring has been established over a considerable period of time, accepted by the Science Board of SRC and a design study costing £70,000 is already well under way.

Storage ring facilities being built in Hamburg and Paris are such that synchrotron radiation users will have limited access and remain parasitic. What is envisaged in the United Kingdom is a unique facility, with 10 access points and accommodating 30 experiments simultaneously. Many experiments which could not previously be performed due to lack of intensity now become feasible. New experiments utilising the polarisation properties of synchrotron radiation have been proposed, and the enormous flux in the 1 Å region will allow time resolution of milliseconds or better in the study of complex biological functions, such as muscle flexing. The storage ring is also expected to be a potent source for scattering experiments below 1 Å.

What the scientists present at Reading were extremely concerned about was the time scale involved in building a dedicated storage ring. The building of such a facility could in principle be started almost immediately because no major new technological advances are envisaged (I omit consideration about the “wigglers” required to reach the shorter wavelengths). It need not be built at Daresbury and therefore need not await the closure of NINA. This would ensure that the United Kingdom has its share of new and exciting physics. Is it realistic, however, to suggest such an alternative? The estimated cost of £2

million would raise, since a new building to house the storage ring, and all the services available in the NINA facility, would have to be provided. Incidentally, Professor Bleaney’s suggestion of a disastrous 5 year period between NINA closure and storage ring commissioning was refuted by Daresbury personnel present. A preliminary estimate is 12 to 18 months, given the level of funding shown in the SRC document. This could be reduced with sufficient manpower and money if SRC were to give whole-hearted support to the proposal.

It is now up to the SRC to act swiftly and decisively in this matter or the United Kingdom will once again find itself an also-ran in the race for scientific discovery. The critics have had their chance to comment on the proposal and no substantial argument was forthcoming. (For example, nobody has suggested that a tunable soft X-ray laser will turn such a facility into an under-used white elephant.) In the absence of such criticism, let us press ahead with a facility which will surely be the envy of synchrotron radiation users throughout the world.

Yours faithfully,

K. CODLING

*J. J. Thomson Physical Laboratory,
Whiteknights, Reading RG6 2AF*

We have also received another letter in similar vein from seventeen of those at the Reading meeting.—ED.

Penicillin

SIR,—I am grateful to Ernst Chain for his comments on the rediscovery of penicillin (*Nature*, June 14). There would, however, seem to be ample justification in the literature for the viewpoint I put forward in my earlier article (*Nature*, May 24) concerning the direction of research into antibiotics during wartime and the quest for agents of chemotherapy. Chain’s first joint paper in the field¹ refers to chemotherapy in title and substance, and his later joint publication that same year (on penicillinase) discusses the relative merits of penicillin as an agent of chemotherapy². The extensive paper on therapy using penicillin published some months later by Chain and the other members of the Oxford team³ expands on the theme. The effects of the war effort, to which I have referred elsewhere⁴ is repeatedly emphasised in Fleming’s classic volume⁵

(to which, apparently, the Oxford workers declined to contribute). Bacharach and Hems⁶ in particular, state that penicillin seemed to “merit much more attention than it had received”, a pointer to exactly the form of directed research I would like to see in oncology. It contrasts radically with Coghill’s comments⁷ that Raistrick, apparently the first to see the significance of antibiotics, “could get no clinical tests made”. Perhaps the essence of what we should say is that, though it is widely assumed that Fleming’s fortuitous discovery gave rise to the concept of antibiosis, the discovery of *Penicillium notatum*, and the first recorded examples of ‘microbial therapy’, it did not in fact originate any of these⁸. In this respect the research work of Howard Florey and Ernst Chain, with their coworkers, was of peerless importance.

Yours faithfully,

BRIAN J. FORD

Cardiff

¹ Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., and Sanders, A. G., *Lancet*, ii, 226–228 (1940).

² Abraham, E. P., and Chain, E., *Nature*, **146**, 837 (1940).

³ Abraham, E. P., Chain, E., Fletcher, C. M., Florey, H. W., Gardner, A. D., Heatley, N. G., and Jennings, M. A., *Lancet*, ii, 177–189 (1941).

⁴ Ford, Brian J., *History of the Second World War*, 2851–2857 (Purnell, London, 1968).

⁵ *Penicillin, its practical application* (edit. by Fleming, A.) (Butterworth, London, 1946).

⁶ Bacharach, A. L., and Hems, B. A., *ibid.*, 24–45.

⁷ Coghill, R. D., *Chem. Engng News*, **22**, 588.

⁸ Ford, Brian J., *The Revealing Lens: Mankind and the Microscope*, 130–135 (Harrap, London, 1973).

Wursted

SIR,—We are not in complete agreement with Neidle’s point (*Nature*, **249**, 212; 1974) that there is a cultural bias in the understanding of our “hotdog” model for repressor-operator interaction. The Germans, for example, might consider it the *Wurst* model of operator-repressor binding.

Yours faithfully,

T. A. STEITZ

B. ENGELMAN

Yale University,

New Haven, Connecticut 06520

The briefer the letter the better its chance of being published. We reserve the right to cut correspondence.

news and views

Ophiolites and oceanic lithosphere

THE most obvious way of testing whether present-day ophiolites represent ancient oceanic crust and uppermost mantle is to compare the properties of such sequences with those of modern oceanic lithosphere. The scope here is wide; and comparisons have already been made in terms of geology, petrology, chemistry, magnetic properties and seismic velocities. Unfortunately, however, the results of these attempts at correlation have not been entirely conclusive because comparisons have proved to be less straightforward than had originally been hoped. No doubt it is easy to be wise after the event; but it is clear in retrospect that some of the, as yet incompletely fulfilled, hopes expressed in the early literature of the subject were based on overly-naïve expectations even if it must be admitted at the same time that some of the subsequent investigations have been afflicted by peculiarly bad luck. But be that as it may, the fact is that comparisons between ophiolite suites and modern ocean floor are likely to encounter some quite obvious difficulties even if it is assumed that present and past oceanic lithosphere were produced by comparable processes.

Problems arise partly because even if ophiolite sequences were submarine in formation they are now subaerial in fact; and so comparison with modern ocean floor means comparison between structures under quite different circumstances. The effects of differing conditions become quite evident when, for example, Matthews *et al.* (*Nature*, **231**, 200; 1971) measured seismic velocities in the outcrops of the Troodos Massif of Cyprus in the hope that they would be similar to the velocities in the layers of the oceanic crust. In fact, the velocities determined were "surprisingly low"—so low that almost all of them, including those in the ultramafics at the bottom of the sequence, were comparable to those in oceanic layer 2 alone. The conclusion that Matthews and his colleagues came to was that the low velocities were probably due to the presence of open cracks in the rocks under very low confining pressures—cracks which are presumably not present in *in situ* oceanic crust, at least in the lower layers. This view later received convincing support from Poster (*Nature phys. Sci.*, **243**, 2; 1973) who showed that ultrasonic velocities are higher in Troodos samples under pressures of up to 2 kbar.

Then there are the more direct effects associated with older material which has been uplifted and simultaneously subjected to considerable tectonic forces. Irreversible chemical changes may have taken place in some or all of the ophiolite components, making direct comparison with newer oceanic material impossible; and physical changes may have made it difficult to measure accurately even the simplest of parameters such as the thicknesses of the various ophiolite units. For example, although Poster found that the seismic velocities in Troodos samples increased with increasing pressure and thus approached more closely the velocities observed in oceanic crustal layers, he also found that even under pressure the velocities in the ultramafics forming the lowest layer of the ophiolite sequence were lower than those in the gabbros comprising the next layer up. Insofar as the velocities in modern oceanic layers in-

crease with depth, the comparison between the Troodos sequence and present-day ocean floor cannot therefore be considered entirely satisfactory. Poster's suggested explanation of this apparent anomaly was that the ultramafics have undergone a considerable degree of serpentinisation, a process known to lower grain density and consequently seismic wave velocity; and a study of the ultramafic samples in thin section confirmed that serpentinisation had indeed taken place.

So far, however, whenever there has been a discrepancy between a property of ophiolite suites and the corresponding property of oceanic lithosphere, it has seemed quite natural to suppose that the 'problem' originates with the ophiolites. Thus it was entirely reasonable for Poster to suppose that if the Troodos Massif is a remnant of Mesozoic ocean floor and yet exhibits an important difference from modern oceanic lithosphere, then something must have happened to the Troodos Massif. But what about the other half of the comparison? Up to now, it has apparently never occurred to anybody to question whether or not ophiolite sequences are actually being compared with the correct model of the ocean floor. On the face of it, of course, there is no reason why anybody should; the generalised oceanic crustal section proposed more than 10 years ago by Raitt (in *The Sea*, Interscience, 1963) has long been a part of the accepted wisdom of geophysics. But on page 136 of this issue of *Nature*, Moores and Jackson present a comparison between certain features of ophiolites and ocean floor which is apparently much more satisfactory than any proposed hitherto—and they do so on the basis not of the Raitt model but of a somewhat different structure proposed about 3 years ago in a little-noticed article by Sutton *et al.* (in *The structure and Physical Properties of the Earth's Crust*, American Geophysical Union, 1971).

What is still generally regarded as the standard model of 'typical' oceanic crust and uppermost mantle (that is, of ocean basins at normal depths away from ridges, trenches, seamounts and so on) comprises three layers below the unconsolidated sediments (layer 1). The values quoted for thickness and seismic velocity differ between authorities; but as originally given by Raitt, layer 2 has a thickness of 1.7 ± 0.8 km and a P wave velocity of 5.1 ± 0.6 km s⁻¹, layer 3 is 4.9 ± 1.4 km thick but possesses a much less variable velocity of 6.7 ± 0.3 km s⁻¹, and layer 4 (uppermost mantle) has a velocity of 8.1 ± 0.2 km s⁻¹. Layer 3 is usually regarded as the bottom layer of the crust, although from time to time some workers have interpreted marine seismic profiles as indicating the presence of a layer with a velocity in the range 7.1–7.6 km s⁻¹. This 'extra' layer has sometimes been described as layer 3 with anomalously high velocity and sometimes as mantle with anomalously low velocity (and even, on occasions, as the result of misleading data)—reasonable interpretations in most cases because either one or both of the 6.7 km s⁻¹ and 8.1 km s⁻¹ layers were apparently missing and/or the relevant profiles were obviously associated with a prominent feature such as a ridge or trench. In short, the intermediate velocities derived from marine profiles were generally dismissed as atypical and not very important.

But Sutton *et al.* came to a quite different conclusion. Using a new method of seismic refraction profiling involving a single ship, sonobuoys, a repetitive seismic energy source and continuous recording (the Asper method), they were able to identify a high velocity basal crustal layer

($7.4 \pm 0.3 \text{ km s}^{-1}$) at eight Pacific Ocean sites ranging from Fiji to the coast of California. Moreover, they were able to find in the literature reports of ten other sites in the Pacific, the Atlantic and the Mediterranean at which a layer of velocity about 7.4 km s^{-1} was sandwiched between a crustal layer of at least 6.0 km s^{-1} and an upper mantle layer of at least 7.7 km s^{-1} . In addition, there are numerous sites at which the 7.4 km s^{-1} layer had been identified but at which either layer 3 or the uppermost mantle had been missed or (in the case of the mantle) inferred from other data. As a result, Sutton and his colleagues proposed that a basal crustal layer about 3.1 km thick, far from being rare in the oceans (though quite familiar beneath continents), exists under all Pacific basins and probably under basins in other oceans as well. The reason why it is frequently missed even by more elaborate refraction techniques, they suggested, is that it appears largely as second arrivals masked by other arrivals. In other words, the situation is analogous to that in which difficulties were encountered in observing layer 2 during the early days of marine seismic refraction work; layer 2 only came to be observed regularly when conditions were arranged for a specific search.

When Sutton and his colleagues first presented their work at a conference at the University of Colorado in 1970, A. Hales praised them for their courage in reporting "some rather unusual mantle velocities" and, by implication, in proposing an important modification to a model which has come to be accepted as one of the near-certainties of geophysics. Since then, the article in which the results were described in greater detail has apparently received little attention. Moreover, even though confirmation of the basal crustal layer would, in the words of Sutton *et al.*, "have major significance for both interpretation of other geophysical data (gravity and surface waves) and speculations on the geologic processes that resulted in the formation of the Earth's crust", the possibility of such a layer has been little discussed. Presumably this is in part because the Asper technique is not yet in widespread use, so that few new results have been obtained. In the meantime, however, Sutton and his colleagues may take heart from the indirect evidence adduced by Moores and Johnson, for if the latter authors are right in their interpretation, the consistency between the Sutton model and the view of ophiolite suites as oceanic lithosphere brings support for both.

PETER J. SMITH

Picornaviruses still useful as model systems

Just as the drift of molecular biology towards eukaryotic systems led to a return-to-*E. coli* backlash, so too the present trend of many virologists towards tumour virology led to the retort "polio is not dead" (Baltimore, *Perspectives in Virology*, 7, 1; 1971). Indeed, as model systems the picornaviruses have much to offer, and still produce regular surprises.

Porter, Carey and Fellner (*Nature*, 248, 675; 1974) have recently demonstrated the presence of a large poly(C) tract, about 90–100 residues long and with 85–90 C residues, within the RNA of encephalomyocarditis (EMC) virus. Since the molecular weight of the viral RNA exceeds by about 1,000 nucleotides that necessary to code for all the viral proteins, and because none of the known viral proteins is rich in proline, Porter *et al.* argue that the poly(C) tract is probably near to one of the termini of the molecule, in the untranslated regions. It is interesting that partially purified EMC RNA polymerase will synthesise poly(G) using exogenous poly(C) as a template (Rosenberg, Diskin,

Oron and Traub, *Proc. natn. Acad. Sci. U.S.A.*, 69, 3815; 1972) and this perhaps indicates a role for poly(C) in replication. Further support for this idea is given by the fact that bacteriophage Q β replicase will also transcribe poly(C) but cannot recognise other homopolymers. Q β RNA does not contain a poly(C) tract, however, and so the significance of these observations remains unclear.

Porter *et al.* have not been able to detect poly(A) in EMC RNA. A similar result was previously reported for the closely related cardiovirus, Mengo (Miller and Plagemann, *J. gen. Virol.*, 17, 349; 1972), but is in contrast to results with poliovirus, an enterovirus, which contains a poly(A) region of 50–100 residues at its 3' terminal (Yogo and Wimmer, *Proc. natn. Acad. Sci. U.S.A.*, 69, 1877; 1972). The reason for this difference between the RNAs of different picornavirus subgroups is puzzling, particularly since it is known that the proteins coded for by the various viral RNAs and their gene order along it are very similar. An understanding of the requirement for poly(A) in the messenger RNAs of viruses which replicate in the cytoplasm might give some clue as to the role of this structure in normal mRNA; on the other hand it is possible that the poly(A) in viruses has a different function to that of the host, for example in viral assembly.

Many plant viruses contain a transfer RNA-like structure covalently linked to the genomic RNA, which can be enzymatically aminoacylated. The tRNA-like structure is present at the 3' end of the RNA, and is different in primary sequence to the corresponding host cell tRNA molecules. The function of these structures is unknown but they are able to interact with several of the enzymes involved in protein synthesis. Salomon and Littauer (*Nature*, 249, 32; 1974) have now reported that mengovirus RNA can be acylated with histidine, by aminoacyl synthetases from mouse liver. Similar enzymes from *E. coli* were unable to produce this result. That the material acylated by the liver enzymes was not contaminating host tRNA was shown by polyacrylamide gel electrophoresis under denaturing conditions. Although cleavage of the viral RNA occurred during aminoacylation, the viral fragment containing labelled histidine was significantly larger than host cell histidyl-tRNA. It is not yet known whether cleavage of Mengo RNA is necessary for charging with histidine or is an artefact caused by contaminating RNases.

Since poliovirus RNA terminates in poly(A) at the 3' end, it cannot have a tRNA-like structure in that position, and not surprisingly previous attempts to aminoacylate polio RNA have failed (Oberger and Philipson, *Biochem. biophys. Res. Commun.*, 48, 927; 1972). This observation therefore serves to emphasise the differences that I have mentioned between the RNA of the cardio and enteroviruses within the untranslated regions.

Earlier experiments have tended to support a role for aminoacyl-viral RNA in protein synthesis. Indeed, the 3' terminal portion of turnip yellow mosaic virus (TYMV) RNA donates valine into polypeptide in a cell-free system (Haenni, Prochiantz, Bernard and Chapeville, *Nature new Biol.*, 241, 166; 1973). The physiological significance of this finding, however, has been questioned by Shih, Kaesberg and Hall (*Nature*, 249, 353; 1974) who made use of the fact that brome mosaic virus RNA can act as a messenger *in vitro* to give well-defined virus-coded products and can also be aminoacylated with tyrosine. Both these activities were examined after chemical modification of the 3' terminus of the viral RNA. As expected, such modification completely abolished the ability of the RNA to accept tyrosine, but surprisingly had virtually no effect on the protein synthetic activity of the viral RNA nor on the authenticity of the cell-free product. Thus Shih *et al.* feel that this eliminates any role for aminoacyl-viral RNA as an obligatory requirement for viral protein synthesis, and instead favour a regulatory role for the tRNA-like structure.

Since protein synthesis elongation factor 1 recognises aminoacyl-tRNA and is also involved in RNA synthesis (at least in bacteria) then perhaps the aminoacylviral RNA is somehow used to integrate protein and RNA synthesis in virus-infected cells.

Two technical problems should be borne in mind, however; first, Salomon and Littauer could only charge about 20% of their Mengo RNA with histidine, and, second, cell-free protein synthesis systems are so very inefficient compared with whole cells that any subtle effects which might be caused by tRNA 'modulation' *in vivo* may be completely lost *in vitro*.

ALAN E. SMITH

258,247 dam earthquakes

by David Davies

BUILD a large reservoir and expect earthquakes. This has been a common experience in the past 20 years. The Koyuna Dam in India and the Kariba Dam in Africa both triggered off much seismic activity and there have been many other similar cases. A well-documented example in China is now presented by Shen Chung-Kang in a recent issue of *Scientia Sinica* (17 (2), 239–272; 1974).

The Hsinfengkiang Dam in Kwangtung Province was started in July 1958 and by August 1960 was producing electric power. The reservoir covers 390 km² and impounds 11,500 million m³ of water. The dam is 440 m long and has a maximum height of 105 m. The immediate vicinity was no more seismic before the construction of the dam than any other area in China, where there is, of course, much scattered seismicity.

Within a month of the first impounding of water in October 1959, seismic activity started up and as the water level rose so did the frequency of quakes. Many of the earthquakes could only be detected instrumentally, but a few were felt in the area around the dam—these generally had a surface-wave magnitude of 2 to 3. A set of seismometers was moved into the area to enable accurate locations to be produced and these still operate.

The first earthquakes were in the region of the dam itself, but progressively the activity spread to other regions. Relatively few events were located under the water itself, the majority being within a kilometre of the water's edge on the landward side. Each new rise in water level seemed to stimulate fresh activity; the depths of the earthquakes were typically 4 or 5 km. Finally in March 1962 came a magnitude 6 event within 1 km of the

dam and 5 km deep. The intensity at the epicentre was VIII—a violent shock, but the dam survived since it had been strengthened months previously on the basis of the earlier activity.

In the last 20 days before the main shock there was a marked reduction in activity everywhere in the reservoir region, and those small earthquakes that there were began to move towards the epicentre of the main shock. Since March 1962 aftershocks have continued with characteristics conventionally expected of an aftershock sequence and the activity in the area continues to this day. Up to 1972, 258,247 shocks with Ms greater than 0.2 were recorded.

What causes reservoir earthquakes? The best general explanation put forward so far, which the author also favours, is that water percolates into the underlying rocks and finds its way into groundwater channels and deep fissures. As the seepage pressure rises the water acts as a means of lubricating, as it were, the faults in the region. Thus earthquakes are facilitated as slippages along these faults. Such an explanation in terms of pore interstitial fluids, is increasingly being favoured as a general explanation of tectonic earthquakes.

Tidal drag cannot move plates

from Peter J. Smith
Geomagnetism Correspondent

THE idea that continents might be displaced by tidal forces was discussed at some length by Wegener in various editions of his book *The Origins of the Continents and Oceans*. But ever since Jeffreys (*The Earth*, Cambridge University Press, 1929) showed that the stress over the Earth's surface arising from tidal friction is only of the order of 10^{-4} dyn cm⁻², few people have considered tidal drag a likely driving mechanism for continental drift or plate motions. On the other hand, such a mechanism does seem to be feasible from the energy point of view. Recent calculations suggest that the energy dissipated tidally may exceed 5×10^{19} erg s⁻¹ (Rochester, *Eos*, 54, 769; 1973), of which about 2.5×10^{19} erg s⁻¹ is likely to be the maximum dissipated in shallow seas and of which less than 10^{18} erg s⁻¹ is lost in the solid Earth. Thus, in principle, as much as $2-3 \times 10^{19}$ erg s⁻¹ might be available for driving plates.

Recently, Bostrom (*Nature*, 234, 536; 1971) and Moore (*Geology*, 1, 99; 1973) have revived the tidal drag mechanism, citing in its support such phenomena as the dependence of seismicity on latitude

Healthy carriers of hepatitis B

from Arie J. Zuckerman
Medical Virology Correspondent

NUMEROUS seroepidemiological surveys on selected groups have shown that the prevalence of hepatitis B antigen, a marker of infection with hepatitis B virus, in apparently healthy persons in Western Europe and North America is 0.1–0.6% by comparison with 5–20% in tropical Africa, South East Asia and the Far East (*Tech. Rep. Ser. Wld Hlth Org.*, No. 512; 1973). Although relatively little information is available from serial samples collected over a period of time, which would permit more precise determination of the carrier rate in defined populations, the implications are that there may be tens of millions of individuals throughout the world who carry silently in their serum the hepatitis B antigen. For practical purposes it has been agreed that a persistent carrier state exists in persons in whom the antigen has been detected repeatedly for more than 3 months. The carrier state may be life long; Zuckerman and Taylor (*Nature*, 223, 81; 1969) described the

and the tendency of oceanic ridge segments to lie north-south (with transform faults lying east-west). But Jordan (*J. geophys. Res.*, 79, 2141; 1974) has now carried out a 'simple calculation' showing that although a plate velocity of 5 cm yr⁻¹ would involve the dissipation of energy at a rate of only 0.8×10^{17} erg s⁻¹ (well within the amount available), a torque of 10^{33} dyn cm would be required to maintain it. The actual couple exerted on the Earth by the Moon is probably less than 10^{24} dyn cm, or 9 orders of magnitude too small to allow plate motion if currently quoted values of asthenospheric viscosity are accepted. Tidal forces could produce lithospheric motion, but only if the viscosity of the asthenosphere were to be as (unbelievably) low as 10^{11} poise.

In further support of the view that tidal forces are not relevant to present-day plate tectonics, Jordan cites recent plate models based on the fixed hot spot hypothesis, which suggest that the westward displacement of the lithosphere as a whole is small compared with the relative motions of plates. In the meantime, however, all this leaves open the rather different question of what happens to the balance of the energy released by tidal friction.

case history of a well documented former blood donor who has been a healthy carrier for more than 20 years.

In high prevalence areas, for example in tropical countries, the antigen is detected in individuals of all ages, most frequently in children. The prevalence of antigen in Caucasians living in some tropical areas is higher than those in temperate zones yet it is considerably lower than in the indigenous population. In all regions the antigen is reported to be more frequent in males than in females and in urban than in rural communities. The universal problem which the carrier state poses to blood transfusion and other medical services, to morbidity from liver disease and to health in general, requires no elaboration. Yet the mechanisms leading to the formation of the carrier state are little known.

In a recent study by Gerety and his colleagues (*J. Pediat.*, **84**, 661; 1974), paired samples of serum from 200 children in the United States and 1,165 children from Upper Volta in West Africa and from two Pacific islands (Guam and Tol, Truk Islands) were tested for hepatitis B antigen by radio-immunoassay and for the homologous antibody by passive haemagglutination. The antigen was not detected in any of 100 normal children in the United States but it was found in 5% of 100 children resident in institutions in that country. The antigen was found in 2–10.9% of sera from normal children from overseas. Hepatitis B antibody was not detected in normal American children, but it was present in 9% of American children in institutions, 3% in Guam, 20.3% in Tol and 7% in Upper Volta.

Gerety *et al.* calculated the 'per cent. carriers' according to the formula: antigen carriers/infected group $\times$ 100, where the infected group is defined as children with hepatitis B antigen or antibody, or both. The figures obtained were nil for normal American children, 35.7% for children in institutions, 40% for children in Guam, 35.2% for children in Tol and 28.6% in Upper Volta. The respective figure for American adults, calculated from published reports, is 1.8–3.5%.

Several different mechanisms may predispose to persistent carriage of hepatitis B antigen. It has been suggested that the establishment of a complete or at least a partial immunological tolerance to this antigen is the most likely explanation. A relative immune deficiency, associated with slow maturation of cellular immunity in newborn infants, is one possibility. This mechanism may play an important part in carriers resulting from vertical transmission of hepatitis B virus (*Nature*, **249**, 105; 1974). Gerety and associates (*loc. cit.*) found, however, that the risk

Channelled scablands of Washington



THIS picture shows part of the 40,000 km² 'channelled scablands' of eastern Washington, and is from a new booklet produced by the US Geological Survey (*The Channelled Scablands of Eastern Washington*, US Government Printing Office, Washington DC, 1974, 65c). The geological events leading to the formation of this unique landscape began during the Miocene with the extrusion of up to 3,000 m of basalt lava flows which later tilted and warped and acquired a

30–60 m cover of loess. Then beginning about 100,000 years ago ice lobes from the northern hemisphere glaciation encroached on the lava field, damming rivers with glacial ice and debris and forming large lakes. Finally, 10,000–20,000 years ago the dam containing the largest lake broke and more than 2,000 km³ of water and debris were released within a day or two, carving the surface as observed today.

P.J.S.

of becoming an antigen carrier seemed to be uniform among children ranging in age from 1 month to 15 years. They considered that factors of exposure such as the route and amount of virus may contribute to the disparate chronic infection rates between children and adults. For example, means of transmission of low dose of virus in children, other than by direct skin penetration, might influence the severity of the infection and the immune response, favouring the development of the carrier state, but the available information is incomplete. An intensive immune response may be essential for the elimination of the virus and the prevention of the establishment of an equilibrium between the host and the infecting agent. There are reports in the literature which suggest that adults seem to become carriers more frequently after a mild attack of hepatitis (Barker and Murrey, *J. Am. med. Ass.*, **216**, 1970; 1971; Aach *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1656; 1971; Gocke, *J. Am. med. Ass.*, **219**, 1165; 1972). And, in general, children experience a milder disease with many subclinical infections.

Carriage of hepatitis B antigen has been consistently reported to be more

frequent in males than in females and evidence has been obtained which indicated sex as an independent primary variable factor responsible for a higher prevalence of the antigen in males than in females. Washburn and colleagues (*Pediatrics*, **35**, 57; 1965) studied the sex difference in susceptibility to a number of infections. A significant preponderance of males was found and this was most marked in infancy. The sex difference in susceptibility was postulated to be consistent with a gene locus on the X chromosome which is involved in the synthesis of immunoglobulins. Small differences in the amounts or rates of synthesis of antibody might be responsible for a slightly greater susceptibility to infection among members of one sex. Gerety and his colleagues found that the likelihood of the development of the carrier state in the groups of children they studied did not seem to correlate with the sex or with the geographical origin of the children or with the subtype of hepatitis B virus. What the results did show clearly was that children became persistent carriers more frequently after infection with hepatitis B virus than adults in the United States.

Identification of HO₂

from our Chemical Physics Correspondent

MOST chemists and chemical engineers would feel that HO₂ is one of the commoner and better known of the gas phase free radicals which are common intermediates in so many reactions. Hydrocarbon combustion, both in heating plants and in motor engines, is a major process in which HO₂ is a significant intermediate and many important kinetic rate constants are concerned with its creation and subsequent destruction. However clear cut, physical identification and study have proved remarkably difficult. Even the powerful tools of photolysis and of matrix isolation have found HO₂ to be a slippery customer and ultraviolet, infrared and electron resonance spectra of only indifferent quality and resolution have been obtained. More informative gas phase spectroscopy has been, hitherto, remarkably unsuccessful and this is not for want of much hard and difficult work.

Now, however, the powerful team of Radford, Evenson and Howard (*J. Chem. Phys.*, **60**, 3178; 1974) has obtained a remarkably clear spectrum which must be attributed to HO₂, unless even now this radical has sent a Doppelgänger to confuse us. The authors believe it relates to HO₂ but the evidence is indirect. The spectrum appears from more than twenty likely reaction mixtures including O₂+C₂H₄, O₂+C₂H₂, H₂O₂+F, H+O₂ and from discharges in water vapour. The spectrum near 130 cm⁻¹ vanishes when the H is exchanged for D or ¹⁶O by ¹⁸O, but it remains unaltered when ¹³C is used instead of ¹²C in the reagents. The species is certainly paramagnetic, probably uncharged and seems to contain an odd number of nuclei like H with a spin of 1/2. The evidence is certainly sufficient to convince most readers, including your correspondent, but is not quite at the level of absence of all reasonable doubt that would be required by a murder jury. A particularly important aspect of the present spectrum is its availability as a monitor during the maximisation of the concentration of the concentration and life-time of the HO₂ in future experiments. This will be of interest to research workers in combustion and further details of the rotational spectrum will be eagerly awaited by astrophysicists who would seek HO₂ in interstellar gas by means of its microwave spectrum.

The spectrum was observed with a powerful technique which uses the absorption of the H₂O and D₂O laser lines near 119, 126 and 139 cm⁻¹ (84, 79 and 72 μm). Since these lines do not coincide perfectly with the HO₂ absorption, the latter is modified by a magnetic field up to 2 T (20 k gauss) using the Zeeman effect. This technique, called laser magnetic resonance, is sensitive only to paramagnetic species, so that the many dia-

magnetic species in the reaction stream do not interfere. Using magnetic field modulation and a phase sensitive detection system about twenty lines are easily detected with each laser frequency using separately the laser electric field parallel and perpendicular to the magnetic field, a change which modifies the selection rule on the Zeeman sublevels. Indeed the signal to noise ratio is such that the doublet nature of the lines, due to the proton magnetic moment coupling with the unpaired electron, is clearly resolved with a separation of 1 mT (10 gauss). The radical is close to a prolate symmetric top and has $A \sim 19.4$ cm⁻¹ and $(B+C)/2 \sim 1.26$ cm⁻¹. The rotational quantum numbers N and K provide useful state labels and the transition near 139 cm⁻¹ is assigned to the K 3 $\leftarrow$ 2 and N 19 $\leftarrow$ 18 transition although N 20 $\leftarrow$ 19 is not completely excluded by the evidence. There is a zero field splitting between the $J=N+1/2$ and the $J=N-1/2$ states which is 0.605 cm⁻¹ for $K=2$ and 1.139 cm⁻¹ for the $K=3$ level. The transitions are for the $J=N+1/2$ states.

Vegetation disturbance in arctic tundra

from Peter D. Moore
Plant Ecology Correspondent

THE slow recovery rate of alpine tundra following the disturbance of surface vegetation (see *Nature*, **249**, 690; 1974) is causing concern. A similar, but more complex problem confronts conservationists in arctic tundra regions, where the existence of permafrost within the soil means that thermal equilibrium is a critical factor in determining ecosystem stability.

Dingman and Koutz (*Arctic Alpine Res.*, **6**, 37; 1974) have studied the pattern of frozen subsoil distribution in a region of central Alaska where permafrost is discontinuous. They were concerned in particular with microtopography and the way in which such factors as aspect can influence the net potential radiation input and hence the depth of the active layer (seasonal thaw zone). A rather rough, non-linear correlation emerges between their potential insolation index and active layer depth, and their figures suggest that permafrost occurs where the average annual solar radiation falls below 265 cal cm⁻² d⁻¹.

The anomalous points on the graphs of Dingman and Koutz, which distort some of their regressions, frequently correspond to locations of unusual vegetation type, such as forest cover or exceptionally thick moss hummocks. Evidently the influence of surface vegetation on ecosystem energy balance, particularly on albedo and on latent

heat losses associated with transpiration, must be taken into account.

This aspect has been studied by Haag and Bliss (*J. appl. Ecol.*, **11**, 355; 1974) who have conducted experiments to test the effect of vegetation disturbance upon tundra energy budgets. Damage to surface vegetation and peat on areas used as roads during the winter months resulted in increased surface moisture and therefore in considerable albedo changes (6.1%, compared with 15.2% in control sites); in consequence there was an increase in net radiation input in the road site. In addition, the compaction or disturbance of the surface peat layer led to an increase in thermal diffusivity, as a result of which higher soil temperatures were recorded during the day even at a depth of 50 cm in the road profile. Latent heat losses were greater in the control site, especially in the growing season when actively transpiring vegetation was fully developed and deeper reserves of water were being tapped.

One of the important conclusions of this work is that the use of tundra as a winter road alters the soil thermal régime, resulting in a deeper active layer in summer (about 56 cm compared with 36 cm in control). Similarly other types of disturbance such as fire and oil spills produced deeper active layers (46 cm and 42 cm respectively). Such conditions are conducive to surface instability and erosion. A possible solution to the problem would be the reseeded of disturbed areas to assist surface healing.

Wein and MacLean (*Can. J. Bot.*, **51**, 2509; 1973) investigated the potential of *Eriophorum vaginatum* (cotton sedge) as a recolonist of bare peat and mineral soil and came to the conclusion that the species could be of particular value where mineral soils were exposed and where the soil is permanently saturated. This is precisely the set of conditions found on parts of the winter roads. The seeds germinated best at 25–30° C and had no dormancy problems; when sown in July they were found to be strong enough to withstand the subsequent winter.

Reseeding experiments in disturbed tundra areas are now being conducted, but the initial data of Haag and Bliss suggest that the development of a vegetation cover will be slow; only 20–50% surface cover has been attained after three seasons' growth. Albedo has risen in the reseeded plots, leading to a decrease in the net radiation input, but active layer depth has decreased by only 2 cm (from 52 cm to 50 cm). It would seem that the loss of thermal insulation provided by a peat cover is even more vital for the maintenance of a shallow active layer than is high albedo. The redevelopment of a peat blanket will obviously take a very long time.

Seven years of L-dopa therapy

by Miranda Robertson

NOMINALLY, the symposium held on June 28 by the Parkinson's Disease Society was to mark the fact that it is now 150 years since the death of James Parkinson. What was most clearly reflected in its content, however, was that it is now seven years since the advent of L-dopa therapy, which seemed at one time to promise freedom from parkinsonism but has turned out merely to suspend sentence. This has left pharmacologists to try and find out what to do next and clinicians with the problem of what best to do in the meantime; and that, apart from an historical excursion by J. D. Parkes (Institute of Psychiatry, London), was what the symposium was about.

There has been no radical change in the therapeutic approach to parkinsonism since Charcot first used anticholinergic drugs a century ago. The aim is still to redress the balance between cholinergic and dopaminergic agonists in the basal ganglia, but with the emphasis on supplying the missing dopamine rather than blocking acetylcholine reception. K. Fuxe (Karolinska Institute, Stockholm) favoured ergot derivatives and in particular 2-bromo- α -ergocryptine (CB154) as future alternatives to dopamine derived from L-dopa. The advantages of these dopaminergic agonists over L-dopa would be that they act directly on the dopamine receptor without having to be converted to the active form by enzymes which may be deficient as a result of the same degenerative process that produces the dopamine deficiency. Fuxe, who has tried CB154 on rats with nigro-striatal lesions, is optimistic about the compound whose effects, though weaker than those of L-dopa, are more prolonged. His hope, however, that CB154 might also be free of some of its side effects is not substantiated by preliminary clinical trials, reported by D. Calne (Hammersmith Hospital, London). Functional improvement was assessed blind at between 9% and 18% on average for 20 patients on the drug, the degree of improvement being positively correlated with the severity of the disability. But adverse reactions seemed to parallel those seen with L-dopa therapy.

Amplification of post-synaptic effects through the adenyl cyclase system, which is also under investigation at the Karolinska Institute, may conceivably help with the separation of therapeutic from side effects, although as C. D. Marsden (King's College Hospital, London) pointed out, it is not clear how different the receptors mediating the one are from those mediating the other. In Fuxe's rat model, however,

theophylline (which inhibits phosphodiesterase and thus increases the concentration of cyclic AMP) does seem to increase activity in the denervated striatal neurones, and he believes that it may be possible to use the drug to potentiate the effects of CB154.

All this, however, assumes a functional post-synaptic receptor mechanism, and as M. Sandler (Queen Charlotte's Hospital, London) pointed out, receptor pathology is by no means ruled out. Sandler, who has long held that the dopamine replacement theory of L-dopa therapy is an oversimplification, devoted much of his talk to hauling skeletons out of cupboards. One of them is ignorance of the exact cause of Parkinson's disease, and of the contribution of deficiencies in chemicals other than dopamine to the syndrome. The other is the failure to explain either how L-dopa works or why it stops working. Neither the disease nor dopa metabolism, in short, is understood.

What seems to be missing is a really good animal model for parkinsonism. Sandler stressed that drugs do not always have the same effect on lesioned rats as they do on parkinsonian humans. This is hardly surprising if only because the parkinsonian lesion is most unlikely to be as simple as the destruction of the nigro-striatal pathway, besides which, autopsy brains of parkinsonian patients show evidence (quoted by M. Yahr, Mount Sinai School of Medicine) of continuing degeneration under L-dopa therapy; whereas the surgical lesion does not evolve.

There is also the question of the effect of prolonged administration of drugs. Clinicians repeatedly brought up the need at the very least for studies on the effects of anti-parkinsonian agents over long periods of time. Progressive disease may explain why parkinsonism in due course overcomes L-dopa therapy; but what is the explanation of the simultaneous increase in the dyskinetic side effects of the drug?

Some recent work of H. Klawans (University of Chicago) suggests that the answer may lie in the post-synaptic dopamine receptors. He used amphetamine to stimulate the dopamine receptors of guinea pigs to the point at which the drug produced stereotyped movements, and found that after regular treatment over a period of time, the movements could be elicited not only by progressively lower doses of amphetamine but also by the dopaminergic agonist apomorphine.

The meeting ended with a review lecture by A. Carlson (University of Goteborg), whose efforts in establishing dopamine as a neurotransmitter in the late 1950s helped to make possible the introduction of dopaminergic antagonists in the treatment of parkinsonism.

An amoeba becomes a flagellate

from F. E. G. Cox
Parasitology Correspondent

PARASITOLOGISTS are tidy people and like to know how parasites should be classified. The understanding of the correct taxonomic status of a parasite is more than the provision of a handy slot in which to file information; it is the basis of an understanding of the biology of the organism and a point to which related organisms can be referred. From such a reference point stems a knowledge of basic biochemistry, physiology and epidemiology and on these studies the control of the parasite can be based. Parasites, particularly protozoa, are often very specialised and it is not always easy to see their basic structures with the light microscope. During the past decade, however, the electron microscope has been used to solve several taxonomic problems. Partially as a result of such studies the piroplasms and *Toxoplasma* have taken their rightful position within the sporozoan class Coccidia, and the number of discrete taxonomic groups containing a few parasites of uncertain taxonomic position has been diminished.

One of the outstanding taxonomic problems is concerned with an amoeba, *Dientamoeba fragilis*, which lives in the gut of man. It has long been recognised that this binucleate amoeba could be a flagellate but absolute proof has been lacking. Two independent papers in the *Journal of Protozoology* have now supplied this proof. Honigberg and his colleagues (Camp, Mattern and Honigberg, *J. Protozool.*, **21**, 69; 1974) now report that the fine structure of *Dientamoeba* places it among the trichomonad flagellates and this conclusion is also reached by Dwyer (*ibid.*, 139) using immunoelectrophoretic methods to analyse the antigens of *Trichomonas*, *Dientamoeba*, *Histomonas* and *Entamoeba*. This latter work is a culmination of Dwyer's earlier immunological studies on these parasites (*ibid.*, **19**, 316 and 326; 1972). All the evidence now points to common structures and common antigens linking *Dientamoeba* with the flagellates *Trichomonas* and *Histomonas* and there is a clear evolutionary sequence in which the number of flagella are reduced in *Histomonas* and disappear altogether in *Dientamoeba*. Honigberg suggests a revised classification of the Order Trichomonadida in which *Dientamoeba* is placed in the same family as *Histomonas* and this is likely to be generally accepted by protozoologists and parasitologists.

The implications of these studies lie in an understanding of the mode of transmission of parasitic members of the order Trichomonadida, Protective

cysts are unknown in these forms and the parasites have had to evolve unusual ways to ensure transmission. *Histomonas meleagridis*, the causative organism of blackhead in poultry, enters the egg of the nematode *Heterakis gallinarum* (Lee, *Parasitology*, **59**, 877; 1969) and there is abundant evidence to suggest that *Dientamoeba fragilis* of man uses the nematode *Enterobius vermicularis* (Ockert, *J. Hyg. Epidem. Microbiol. Immunol.*, **16**, 213 and 222; 1972; *Abstracts 4th Int. Cong. Protozool.*, Clermont-Ferrand, 1973). As very little is known about the affinities or the transmission of the three species of *Trichomonas* in man there may be some surprises for those who suggest infection as a result of direct contamination.

Structure of nuclear single-particle states

from Peter E. Hodgson
Nuclear Theory Correspondent

MANY features of nuclear structure can be understood by considering the individual motions of the constituent nucleons. For most of the time they move on independent orbits, and occasionally collide with each other. The energies and quantum numbers of these orbits can be determined by reactions that remove a nucleon from a nucleus or add one to it.

The single-particle states near the Fermi surface—those with the highest energies—can be most easily studied by one-nucleon transfer reactions like the (d, p) stripping reaction. Deeper states can best be studied by nucleon knock-out reactions like (p, 2p).

Many studies of single-particle states have now been made, and their systematic behaviour from one nucleus to another is becoming clearer. It is found that most states are split into a number of fragments by the residual interactions that are not taken into account in the simple shell model. Thus instead of a single state several states of the same structure and quantum numbers are spread over one or two MeV of energy. The energy of the state, however, can still be defined as the centroid of the fragments, weighted by their spectroscopic factors.

A very important question is then whether all the fragments of a particular state have been found, for if some fragments far away from the main strength have been missed this could produce serious errors in the centroid energies. It is not easy to detect small fragments experimentally, so other methods must be tried.

If one could rely on the theory of the interaction removing the nucleon there would be no difficulty, for one could simply calculate the sum of the

spectroscopic factors and see if they added up to unity, the value corresponding to a pure single-particle state. Unfortunately, even in favourable circumstances the distorted wave theories cannot be relied upon to better them about 10–20%, and one is concerned with the loss of small fragments constituting around 5–10% of the total strength.

Another approach is to use a sum rule with all the single-particle states in a particular nucleus. Koltun (*Phys. Rev. Lett.*, **28**, 182; 1972) has shown that provided only two-body forces are present in the nucleus, the total energy of the nucleus may be expressed as a sum of the kinetic and removal energies of the constituent nucleons.

$$E = \frac{1}{2} \sum_i (T_i + E_R^i)$$

The total energy E is known accurately from the nuclear masses, and the kinetic energies T_i can be calculated quite easily from a simple nuclear model. The removal energies E_R^i are obtained directly from the centroid energies. The sum rule thus provides a sensitive test of the completeness of the sets of fragments assigned to each single-particle state. It does, however, refer to all the nucleons, so the set of single-particle states is tested as a whole, and not individually.

Since the most complete sets of data are available for the proton single-particle states, it is useful to split the above sum rule into the corresponding relations for protons and neutrons separately. Thus the mean binding energy per proton becomes

$$E/Z = \frac{1}{2} [\langle T \rangle + \langle E_R \rangle - m_p \langle T \rangle / M_{A-1}]$$

where the angular brackets denote averages only over the proton single-particle states. The extra term is a recoil correction that must be included in accurate work.

This sum rule has recently been tested by a group at Saclay (Bernheim *et al.*, *Phys. Rev. Lett.*, **32**, 898; 1974) using the (e, e'p) reaction at 497 MeV. This reaction is preferable to the (p, 2p) reaction because the electrons are less likely than protons to interact with other nucleons before or after their main quasi-elastic interaction with one of the nuclear protons. For the same reason the cross section for the knockout reaction is reduced, but improvements in the experimental apparatus have so increased the incident intensity that it is possible to obtain significant results in a practicable time.

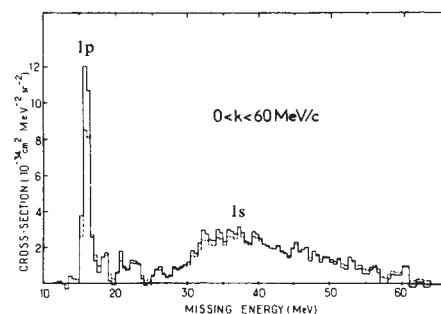
The single-particle states are detected by measuring the energies of the inelastically-scattered electron and of the ejected proton. Application of the conservation laws then gives the energy required to remove the proton, which is just the required energy of the

single-particle state. If the distribution of this energy is plotted for a large number of knockout events the single-particle states are evident as peaks, and the angular distributions of these peaks enable the quantum numbers of the corresponding single-particle states to be found.

The results for $^{12}\text{C}(e, e'p)$ are shown in the figure, and clearly show the peaks corresponding to proton removal from the 1s and 1p states. The widths of these peaks are due partly to the inaccuracies in the measurements and partly to the energy spread of the fragments which are not resolved individually.

These data were used to test the proton sum rule. After correcting for distortion of the outgoing proton waves in the nuclear field they found -4.0 ± 0.5 MeV for the mean binding energy per proton, compared with the value -6.93 MeV obtained from the nuclear masses with Coulomb corrections. This is a very significant discrepancy. One possible explanation is that there are some small fragments many MeV away from the main peaks. Bernheim *et al.* estimate that 5% of the strength associated with average separation energies of 150 MeV would be sufficient to account for the discrepancy. The rate of fall-off of the 1s peak with increasing energy is such, however, that if it continues to higher energies this explanation is very unlikely. On the other hand if there is a discontinuity in the fall-off this constitutes a new phenomenon requiring explanation.

Another possible explanation of the discrepancy is the presence of three-body forces, whose absence was assumed in deriving the sum rule. In order to account for the data, the contribution of three-body forces to the binding energy per proton would have to be $\langle H_3 \rangle / Z = 5.9$ MeV, which at present seems unlikely. If this possibility is confirmed, it would be of



Spectrum of the 'missing energy' in the reaction $^{12}\text{C}(e, e'p)$ with (—) and without (---) radiative corrections. The 'missing energy' is equivalent to the energy required to remove a proton from ^{12}C , and the peaks at about 16 and 35–40 MeV correspond to removal from the 1p and 1s states respectively.

great importance for the study of nuclear structure. It is also possible that the discrepancy would be reduced or even eliminated by a full relativistic analysis, but this has not yet been done.

Sun-shuttling lizards

from our Animal Ecology Correspondent

LIZARDS are distinctive by their relative absence from temperate forests. This situation, on the face of it, seems anomalous in view of the great abundance of insect prey species in this sort of habitat. Huey (*Science*, **184**, 1001; 1974) now points out that this anomaly may be resolved by considering the ecological difficulties encountered by poikilothermic carnivores.

Unable to thermoregulate physiologically, most lizards move about from sunshine to shade or modify their body posture to alter surface areas exposed to heat sources and thus thermoregulate behaviourally (Cowles and Bogert, *Bull. Am. Mus. nat. Hist.*, **83**, 261; 1944; Heath, *Univ. Calif. Publ. Zool.*, **64**, 97; 1965). Their thermal strategy can be described as heliothermy. It is theoretically possible to predict the amount of behavioural thermoregulation that would maximise net profits by calculating the 'costs' of a physical movement into or out of sunshine as against the 'gain' involved in the attainment and maintenance of a specific body temperature. In Huey's study on *Anolis cristatellus* in Puerto Rico the energetic cost of physical movement from shade into sunshine was estimated by measuring the shortest practicable distance from a perch in the shade to one in the sunshine. Anoles occupy a variety of habitats. Huey chose to study two adjacent populations, one in a shaded forest and the other in an open meadowland. If a tree or other perch was totally shaded and offered no sunlit patches, this fact was noted. Many more lizards in the forest were unable to reach full sun without changing perches (which usually meant changing trees) than were those in the meadow. Furthermore, those lizards in the forest that could reach the sunshine were significantly further from it than were those in the open. Thus there seemed little doubt that the energetic 'cost' of heliotactic thermoregulation was far higher in the forest population.

During the early morning the cloacal temperature of anoles in the forest increased significantly slower than that of meadow lizards and was strongly correlated with ambient air temperature. Such a strong relation did not exist between lizards living in the open and the surrounding air; body temperature of lizards in the open fluctuated by only 1.6° C during the day as compared with 4.9° C in the forest.

The interpretation of these observations is that behavioural thermoregulation occurs only where sun and shade conditions are juxtaposed. Where 'costs' are high, as in the three-dimensional environment of the forest, anoles are passive to ambient conditions. Under these conditions the rewards of sun-shuttling are insufficient and this is reflected in very low population densities. What is the evolutionary significance of this exploitative strategy?

A. cristatellus is a remarkable reptile in having such a plastic ecology. The genus is known for its ability to colonise islands *de novo* as well as to form sympatric species on islands already colonised (Williams, *Q. Rev. Biol.*, **44**, 345; 1969), and its degree of thermal tolerance possibly accounts for its widespread success in the Caribbean. Those populations of *A. cristatellus* that occupy open habitats have an energy budget from which reproduction can have a larger share than is possible in forest populations. Although lizards in the two habitats are indistinguishable in terms of dewlap colour, snout-vent length or midbody scale rows, and do not differ with respect to preferred body temperature in a laboratory gradient, it would seem likely that their populations are in the process of active divergence. Perhaps sun-shuttling is the key to two questions: first, the absence of lizards in forests; and second, the complexity of *Anolis* speciation.

Magnetospheres of Earth and Jupiter

from N. A. Heard and A. R. L. Tatnell

THE tragic death of Neil Brice earlier this year in an air crash over the Pacific led to the renaming of the symposium in Frascati (May 28–31) on the magnetospheres of Earth and Jupiter, the Neil Brice Memorial Symposium. After V. Formisano (Spazio Plasma Laboratory, Frascati) had opened the meeting, C. Kennel (University of California) described the valuable contribution of Neil Brice to the study of the Earth's magnetosphere.

The symposium fell naturally into two parts. In the first half the Earth's magnetosphere was reviewed and results from several satellites were presented. K. Schindler (Ruhr University, Bochum) and V. Vasylinas (Massachusetts Institute of Technology) presented theoretical calculations based on models of the magnetosphere; some attempt was made to extend the model of the Earth's magnetosphere to that of Jupiter.

It has been known for some time that Jupiter is a very intense planetary radio source but, as D. Gurnett (University of Iowa) showed, the Earth is not to be outdone in this respect. Imp-6 and 8

satellites have revealed that the Earth is a comparable radio source and in the frequency range from 50 kHz to 500 kHz, at peak intensity, the total power emitted is about 10^9 W. This radio emission is associated with discrete auroral arcs and seems to originate from altitudes of about 2,000 km. It was suggested that a satellite at this altitude might provide some interesting results.

As R. W. Fredericks (TRW Systems, California) pointed out the value of studies of wave-particle interactions to the understanding of the Earth's magnetospheric processes; it was generally agreed that similar studies of the wave-particle interactions in Jupiter's magnetosphere are essential.

Motion of the Earth's bow shock—the shock caused by the supersonic solar wind impinging on the Earth's magnetic field—was discussed by several speakers. The causes of shock motion are not understood but, from data from Heos 1, Formisano and G. Mastrantonio (Frascati) showed that a satellite is likely to encounter the bow shock in motion if tangential discontinuities are the main cause of motion.

E. J. Smith (Jet Propulsion Laboratory, California) confirmed that the outer boundary of Jupiter's magnetosphere fluctuates considerably, a possibility mentioned by several speakers. The vector helium magnetometer results have revealed that the magnetopause is crossed twice on the inbound pass and a number of times on the outbound pass. An interesting feature of Jupiter's magnetic dipole field is that it is directed opposite to that of the Earth. This dipole field is inclined 15° to the rotation axis and offset about $0.1R_J$ north of the equatorial plane and about $0.2R_J$ towards longitude 170°. From this estimated dipole moment the surface field is between 2.3 and 11.7 gauss compared with the Earth's field of between 0.3 and 0.6 gauss. The dipole approximation for Jupiter is even less valid than in the case for Earth, because of the considerable contribution from current sheets around the planet. Pioneer 10 has also confirmed the presence of helium on Jupiter and the existence of an internal energy source in the planet because the excess radiation is 2 to $2.5 \times$ the solar input.

The role of the Galilean moons is still uncertain but, as several speakers showed, Io in particular seems to influence the particle flux considerably. Io is about the same size as Earth's moon and occultation experiments have shown that it has an ionosphere and a surface pressure of 10^{-6} bar.

The fly-by of a planet obviously leaves a number of unanswered questions and all speakers emphasised the difficulty in distinguishing between spatial and temporal variations.

Health costs associated with the mining, transport and combustion of coal in the steam-electric industry

L. A. Sagan

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By assigning an arbitrary cost to a human life, Dr Sagan arrives at rough estimates of the health costs associated with power generation. The costs of miners' accidents and diseases are shown to be heavy compared with those of air pollution.

CHOICE between energy sources rarely takes into account factors other than the market value of the commodities^{1,2}. The costs of such decisions to society in general are rarely considered. Some of these costs are environmental: for example, disposal of industrial waste has often been free. Others involve the health, both of the general population³ and of the persons employed in the industry of interest. In my recent study of the nuclear fuel cycle⁴ I showed that the health effects incurred by occupational personnel are far greater than those suffered by the public.

In this study I evaluate the health costs of generating electricity from the combustion of coal. The health costs of coal production and combustion should be considered in energy planning, but it would be presumptuous to imply that choices among alternate fuels will be made solely on the basis of health. Even so, such quantitative data as are available, as well as an understanding of the deficiencies of the data, should be available to planners and decision makers.

I decided to consider the use of coal for the generation of electricity for the following reasons. Although energy demands in the United States are generally doubling every 14 yr, demand for electricity is now doubling every 7 yr. Although nuclear fuel is expected to increase its share of electrical generation to 66% by the year 2000, coal production for steam generation will continue to increase (Table 1).

It is difficult to consider together such diverse health effects as accidental death and injury, and chronic disease caused by industrial chemicals, dusts and gases. The latter are elusive, and their existence and magnitude is difficult to demonstrate. The technique I adopted was the assignment of dollar losses associated with disability and death. This requires an arbitrary assignment of a cost per human life. The figure I have chosen is \$300,000 which is the multiple of an assumed productive value of \$50 d⁻¹ and a loss for accidental death of 6,000 working days as suggested by the American National Standards Institute⁵. No attempt is made to put this estimate on an annual basis by future discounting. I must emphasise that this is not an attempt to make moral judgment about the value of life. Such a valuation of life is implicit in decisions concerning

health and safety expenditures. In a study for the Office of Science and Technology⁶, a life value of \$140,000 was assumed with which to measure the cost effectiveness of automobile safety features. This was arrived at simply by multiplying average American per capita income (\$3,786) by the average age of those who died in automobile accidents (36.9 yr). It is also of interest that in a recent analysis of risks and benefits of United States technology, C. Starr (personal communication) has found that the implicit value of life in public acceptance of technology is \$300,000.

My evaluation is limited to effects which investigators have tried to quantify. In general, I have adopted pessimistic assumptions regarding health hazards in order to assess worst possible effects rather than to ignore effects for which there may be some evidence but not demonstrated proof. For example, the possibility of threshold levels of toxicity are ignored in favour of the assumption of the same proportionate effect at low levels of exposure as found at higher levels. This assumption is often made, particularly for radiation effects, but has recently been criticised as being at variance with the available biological evidence⁷. Health costs are evaluated in relation to measures of true or real value rather than in terms of out of pocket expenditures since these health costs have rarely been paid by the industry responsible for the injuries. A cost of \$50 d⁻¹ is assessed for lost time due to accident, and an equal amount for medical costs incurred. This latter assumption is based on an estimate of medical costs of all accidental injuries⁸.

So I have estimated the health costs of the 180 GW generated by coal-fired power stations in the United States. Those costs are then reduced by a factor of 180, to represent the equivalent of a single 1,000 MW (electric) plant which is the size of a modern plant supplying a city of 1 million people.

For a 1,000 MWe electrical generating plant of modern design, about 2 million tons per year of coal are required, assuming a heat efficiency of 8,500 BTU per KW h, a 67% load factor and the use of a medium grade bituminous coal of 12,000 BTU per pound. In 1970, by the Census of Mineral Industries Report, 140,000 persons were employed in production of bituminous and lignite coals⁹. Production was 3,350 tons coal per employee, so to supply a 1,000 MWe plant for one year with coal would require the labour of 600 persons.

Morbidity and mortality

In 1969 the underground coal mining accident frequency rate was the highest among all industries—nearly 32 disabling injuries per million man hours compared to the average for all industries of only about eight per million man hours¹⁰. As productivity has increased with increasing mechanisation, the risk of coal mining, as measured in fatalities per million tons mined has fallen, but coal mining still remains more than twice as hazardous as metal mining and almost three times as hazardous as non-metal mining. In the five years 1966–1970, 966 officially reported underground miners lost their lives, an average of about 200 men annually. Although surface or strip

Table 1 Predicted use of fossil fuels

	Total energy ($\times 10^{15}$ BTU)	Electricity ($\times 10^{12}$ KW h)	Fossil Fuels for electricity ($\times 10^{15}$ BYU)
1970	69	1.5	14
1985	133	4.5	20
2000	191	9.0	24

mining is considerably less hazardous than underground mining, there were also 31 fatal accidents in surface mining in 1969 and 33 in 1970, the total yearly average thus being approximately 230 men. In 1970, 48.3% of coal was mined from the surface and of this 75% was used by electric utilities. Thirty-nine per cent of deep mined coal was used by electric utilities (US Bureau of Mines, personal communication). Based on these estimates, 103 fatalities can be attributed yearly to the 180,000 MWe of coal generating stations. This is equivalent to slightly more than a half a fatality per 1,000 MW electric steam plant of \$171,000 yr.

In addition to fatal injuries, there were about 2,000 d lost per million man hours worked in 1969 and 1970 (ref. 9). Assuming an 8 hour working day, 1 d in 62.5 is lost to accident. For a 1,000 MW plant requiring the labour of 600 men for its fuel supply, 2,880 d at a cost of \$288,000 would be incurred.

The Bureau of Mines has recently initiated a study of cost-effectiveness of mine safety measures which, if not definitive, at least provides a model for future research and development¹¹. A summary of their recommendations with estimates of cost to the industry together with an estimate of lives saved annually is seen in Table 2. These recommendations are not, however, mutually exclusive nor additive. They also ignore savings that might accrue from increased productivity of new techniques, reduced compensation insurance costs, and reductions in non-fatal accidents.

Pulmonary disease

The Federal Coal Mine Health and Safety Act of 1969 establishes pneumoconiosis as a compensable disease occupationally related to coal mining, based on the observation that both morbidity and mortality from pulmonary disease, regardless of cause, is very significantly increased among coal miners¹². Though the relationship between coal dust inhalation and pulmonary disability remains uncertain¹³⁻¹⁵, some estimate of pulmonary disability from coal mining seems in order.

Since no single objective measure of coal-workers pneumoconiosis relates well with clinical disability, miners' complaints of shortness of breath (dyspnea), are the best measure available. Summing the percentage of men in all age groups with severe dyspnea, the increased frequency of disability is approximately $\frac{1}{2}\%$ per year of underground mining experience¹⁴. Most men over 45 with severe dyspnea are disabled, that is, non-working, whereas most men with no or slight dyspnea, both under and over 45, are active. Although this estimate does not fit the data with precision, it does not seem to be an extreme view.

Applying the 0.5% yr formula to the 600 men providing coal to a 1,000 MWe electrical station, 3 men per year would be disabled. Since the Social Security Administration estimates the median age at first application for disability for this disease among miners as 57.1 yr¹⁶, and assuming a normal working life to age 65, then an average of 7.9 yr of working life are lost per man afflicted. Calculating 300 working days per year, each day valued at \$50 d⁻¹, total cost per electrical station per year would be \$355,000.

Table 2 Cost-effectiveness of various improvements in coal mine engineering

Equipment	Annual cost ($\times \$ 10^6$)	Lives saved (yr ⁻¹)
Temporary roof support system	1.62	22
Permanent roof support system	1.5	6
Maintenance and repair	15.2	10
Operator protection on face equipment	1.306	10
Shuttle haulage	40.0	12
Machine loading roof fall accidents	0.045	5
Alternative equipment modifications	(6.611)*	11
Auger-type continuous mining accidents	0.150	6
Performance of miscellaneous hand operations—roof scaling	0.180	8
Surveying	0	1
Brattice maintenance	0.240	3

* Source: abstracted from data in reference 11.

Table 3 Emissions from coal-fired electrical generating stations ranked by effect

Pollutant	Total US emissions (10 ⁶ tons)	Coal-fired steam plants (10 ⁶ tons)	Effect factor		
		(A)	(B)	(A $\times$ B)	%
SO ₂	33.4	15.8	15.3	242.0	62.0
Aerosol	35.2	3.7	21.5	79.5	20.3
NO ₂	23.8	3.1	22.4	69.4	17.7
CO	115.4	0.2	1	0.2	0.1
Total	207.8	23.8		391.1	100.0

Source: ref. 25.

These estimates are based on experience with technology and regulatory standards for dust control which are now replaced by newer mining conditions. But because those characteristics of coal dust which produce pulmonary disease are unknown, there can be no confidence that current dust control standards and procedures will be effective in reducing disease. Furthermore, modern coal mining equipment tends to create more dust, not less.

The Bureau of Labor Statistics does not list accident rates for steam plant operation as a separate category. Information is available, however, from the Tennessee Valley Authority's Colbert and Gallatin plants (personal communication) which both approximate the 1,000 MW plant which serves as my model. Averaging the experience for 1967-71, 6.65×10^5 man-hours were worked annually at each plant with 388.5 d lost because of accidents. There were no fatalities.

Transport of coal

At present 2,300 people are killed annually in the United States by railroad trains, and more than 20,000 persons injured, mostly at grade crossings¹⁷. There were 27,015,000 carloads of freight hauled by railroads in 1970 and 5,296,000 of these were coal. Since half of the coal consumed in the country is destined for steam stations, it can be calculated that about one tenth of all railroad cars are hauling coal to electrical generating plants. If that assumption is correct, then about 230 people are now being killed annually and 2,000 persons injured from transport needs for electrical generation from coal. Since this generation capacity represents the equivalent of approximately 180 stations of 10³ MWe each, 1.3 deaths would occur for each plant. In the absence of any data on the magnitude of disability associated with non-fatal accidents, no charge can reliably be assessed for these losses. Nor is there any charge for other modes of transportation, that is, barge or truck.

Combustion of coal

Goldsmith has reviewed episodes¹⁸ of intense smog which are generally associated with a transient increase in the incidence of death from cardiopulmonary disease among the elderly and chronically ill. This may only reflect a life shortening of a few weeks or months in those already ill, since analysis over several years does not show correlative relationships with air pollution levels. It is more difficult to assess effects on induction of chronic disease from more moderate concentrations of pollutants. Unfortunately, too many other variables have effects which cannot be distinguished from those of the combustion products of fossil fuels (see, for example, ref. 19). There is also the difficulty of identifying the relative effect of each of the air pollutants.

Most attention has focused on sulphur dioxide which can produce acute toxicity in both human and animal populations²⁰. The necessary concentrations are, however, far greater than those found in urban air. Acute smog episodes, such as those of London, 1952 (ref. 2) and Donora, Pennsylvania, 1948 (ref. 22) have produced increased mortality with SO₂ levels which cannot be demonstrated to produce unequivocal human disease.

The earliest published attempt at estimating the cost of health from air pollution is that of Ridker²³ who estimated that 18 to 20% of the approximately \$2 billion in national health costs for respiratory diseases are due to air pollution. Thus the damage to health from air pollution in 1958 was between \$360 and \$400 million.

The Lave-Seskin study³ expands the scope of disease covered by Ridker. In particular they take into account air pollution damages to health in the forms of heart disease and of cancers of the stomach, oesophagus and bladder. Approximately half the lost income and current medical expenses associated with morbidity and mortality from bronchitis are ascribed to air pollution. The coefficient for lung cancer is estimated to be 0.25. In other disease categories air pollution is held responsible for an estimated 15% of the damages associated with non-respiratory lung cancers, 25% of all respiratory diseases and 10% of cardiovascular diseases. These coefficients were estimated by regressions that were run on data from published epidemiological studies.

The total annual reduction in health costs which Lave and Seskin anticipate from a 50% reduction in air pollution is \$2,080 million. Scaling up to 100% implies a total cost of \$4,160 million annually. An Environmental Protection Agency evaluation²⁴ has updated this figure calculated in 1963, to \$6,060 million to account for inflation.

The method of regression analysis as a technique for assessing health effects of air pollutants is not definitive; but in the absence of other data I shall use this value of \$6,060 million for evaluating health costs of coal-combustion products.

Of the total tonnage of United States air pollutants shown in Table 3, coal burned for electrical generation is responsible for approximately 8% (ref. 25). To apply this percentage to the total air pollution health cost of \$6.06 billion would be to treat all pollutants as having equally damaging effects on a weight basis. Walther²⁶ has calculated an 'effect factor' for each pollutant on the basis of its toxicity as reflected by primary air quality standards. The technique is obviously crude, but the health impact of any pollution source can be roughly estimated by multiplying the annual emission of each pollutant by the effect factor. This (Table 3) produces an annual estimate of \$357.5 million for the nation as a whole or approximately \$2 million per 1,000 MW plant. Such an evaluation would clearly be high because of the implicit assumption that all emission sources contribute equally to ground level concentrations, and ignores the fact that the use of high stacks at steam plants very much increases dispersion and therefore reduces ground level concentration.

British studies demonstrate that even in the neighbourhood of a modern 1,000 MW electric plant less than 5% of ground level SO₂ can be attributed to plant emissions. This was equivalent to adding only 0.1 to 0.2 parts per hundred million (p.p.h.m.) SO₂ to average background levels measured at the worst point. At greater distances where population density would usually be greater than in the immediate plant area, ground concentrations of SO₂ contributed by the plant would be in the range of 0.01–0.02 p.p.h.m., which is in the order of 1% of background levels. SO₂ generated by steam plants

cannot be detected beyond 20 mile even under the worst meteorological conditions. Nevertheless, I shall arbitrarily accept the value of 1% of the \$357.5 million cost estimated from the tonnage of pollutants emitted from coal-steam plants. This produces an estimated total cost for the 180,000 MW of coal-generated electric power of \$3.6 million (\$20 thousand per 1,000 MW electric plant).

Wilson has estimated from Norwegian data a damage function for ambient SO₂ levels of 10,000 deaths per million "man-concentrations" (a man concentration is equal to number of persons exposed, multiplied by average exposure to SO₂ in p.p.m. averaged over one week)²⁸. Estimating the population within 20 miles of a steam plant from recent environmental impact statements, it would appear that 400,000 people live within this vicinity. Exposure to 0.02 p.p.h.m. for one year would produce, on the basis of the Wilson estimate, 0.8 death.

Although the evidence relating the health effects of air pollution to its sulphur dioxide content is frail, environmental control efforts focusing on reduction of ambient sulphur dioxide levels have been adopted with little opposition. Techniques for the removal of SO₂ from stack gases are still relatively unproven; however, the US Bureau of Mines estimates that for the technique receiving the greatest interest commercially, lime/limestone scrubbing, costs (including capital charges) are in the range of 1.0 to 0.25 cents per kW h. This system has not yet proved capable of removing more than 30 to 50% of the sulphur dioxide present. At best, stack gas removal systems are not expected to remove more than 75% of the SO₂ present. For most United States coal, this degree of removal would not be sufficient to reach current ambient air standards²⁹. Clarke, Lucas and Ross³⁰ have calculated costs of reducing ground level concentrations from a coal-burning steam plant (Fig. 1). They conclude that stacks are far more economical than sulphur removal systems for reducing ground level concentration.

Adopting the meteorological and cost assumptions³⁰, shown in Fig. 1, and the damage function of Wilson (1 death per 100 man-concentrations) Table 4 was constructed. A population density of 320 persons km⁻² was also assumed producing an expectation of 42 deaths annually with a stack height of 30 m. Intuitively, this result clearly seems unlikely, probably implying a highly inflated risk factor.

With ground level concentrations converted to mortality data, an assumed value of life can be applied to evaluate cost-effectiveness of reducing sulphur dioxide concentrations. The precision of the numbers may give the illusion of precise knowledge which is, in fact, not available.

Accepting the life value used in this study (\$300,000) or even a far smaller one, tall chimneys are clearly cost-effective, whereas sulphur removal processes seem relatively cost-ineffective except in the unlikely case of sources near ground level. This implies that scarce, low-sulphur fuels should be reserved for ground level sources whereas tall stacks should be used for major industrial sources such as steam plants. This conclusion ignores questions of property damage or possible benefits to agriculture from sulphur contamination.

Total human costs of operating a 1,000 MW station are summarised in Table 5. Although these costs are indeed large

Table 4 Cost effectiveness of stacks and sulphur removal in reducing mortality

Chimney height (m)	Capital cost (\$)	Chimney alone		Plus 50% S removal (\$5,000,000)		Plus 75% S removal (\$10,000,000)	
		Annual deaths	Implicit life value* (\$)	Additional lives saved	Implicit life value (\$)	Additional lives saved	Implicit life value (\$)
30	0	42	0	21	238,000	31.5	318,000
70	120,000	23	222	11.5	434,000	17.3	578,000
100	333,000	11	358	5.5	910,000	8.25	1,220,000
120	575,000	4	504	2	2,500,000	3	3,330,000
170	1,600,000	0	1,270	0	infinite	0	infinite

* Implicit life value = total reduction in mortality over 30 yr plant life divided by capital cost.

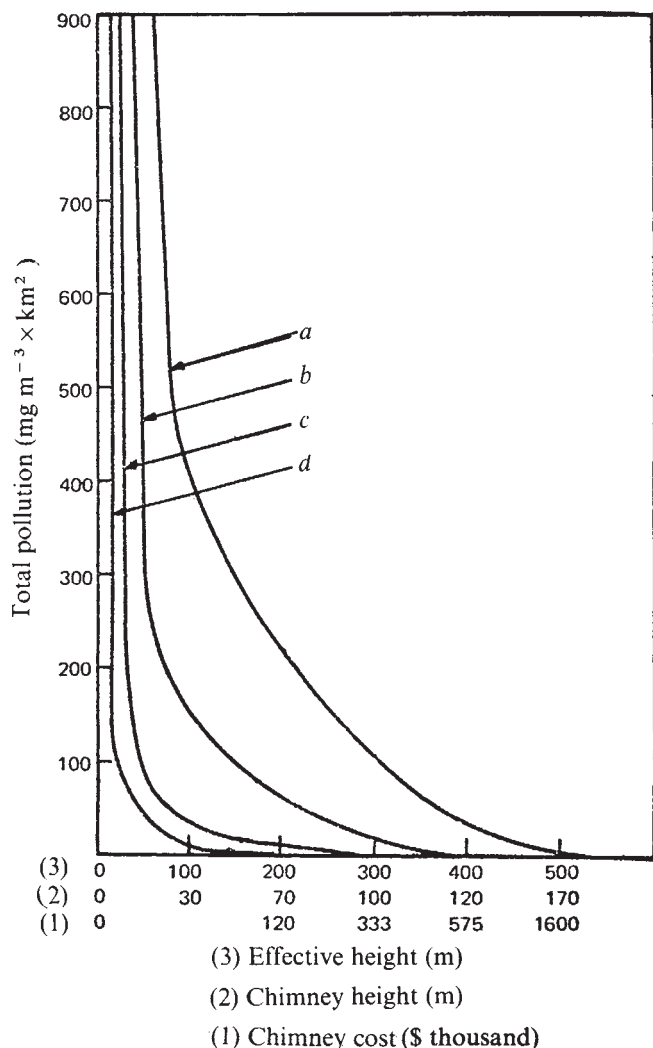


Fig. 1 Dependence of total pollution on source strength and chimney height. Threshold: $100 \mu\text{g m}^{-3}$; wind speed: 5 m s^{-1} . Source strength of SO_2 (kg s^{-1}) and capitalised cost of its removal: a, 1, \$0; b, 5, \$ 5 million; c, 25, \$ 10 million; d, 125, \$ 15 million.

in human terms, they are small compared to annual revenues generated by such a plant. Assuming a load factor of 67% and sales at 2 cents per kW h, annual revenue from such a plant would be about \$29 million.

Mining of coal is seen to account for approximately 50% of all health costs associated with the generation of electric energy from coal. Strip mining is only about 15% as likely to cause death and probably provides a similar advantage in terms of coal workers' pneumoconiosis. This phenomenon receives little attention from those who are attempting to limit or eliminate surface mining in favour of the far more hazardous

Table 5 Summary of annual health costs for 1,000 MW coal-fired electrical station

		\$
Miners	Deaths	171,000
	Accidents	288,000
	Pneumoconiosis	355,000
Transport	Deaths	380,000
	Accidents	*
	Station operation	38,850
	Air pollution	20,000
	Total	1,252,000

* No estimates available.

underground mining. Still, opportunities for accident reduction in underground mining which are highly cost-effective seem to be available but are so far unexploited.

On the other hand, air pollution costs seem to be small compared to other health costs of coal-steam plants—less than 5%. Sulphur removal techniques at their present state of development cannot be shown to be cost-effective, particularly in contrast to stacks and other operational control procedures. Because of the greater effectiveness of the latter, regulatory control would rationally relate to ambient air standards where the operating utility could avail itself of cost-effective techniques rather than to effluent standards where the utility must resort to technology which is clearly cost-ineffective. Furthermore, ambient controls are more likely to reduce levels of other pollutants a corresponding amount whereas effluent controls are likely to be specific to SO_2 . Since the health hazard of air pollution from the combustion of coal is not known to be related to SO_2 exclusively, the importance of this effect is obvious.

It may be useful to compare this estimate of health costs with my estimates for a nuclear power plant of equal power rating⁴. Total costs for 10,030 MWe of the current United States nuclear industry were evaluated at \$2,175,520. For a single 1,000 MWe nuclear plant, cost would be \$217,552 or less than 20% of the annual cost of the coal fuel plant. Although there are uncertainties in both estimates, it would seem likely that the nuclear plant is clearly less hazardous than the coal-fired plant. An official Swedish study³¹ comparing nuclear plants with oil fuel plants reached essentially the same conclusion although the spread between the two fuel sources was found to be considerably greater. Lave has reached a similar result³².

These conclusions refer to normal operating conditions and ignore possible differences in accident potential, for which precise data are not available. Recently Starr *et al.*³³ have analysed and compared accident probabilities for nuclear plants and fossil-fuelled generating plants. They conclude that the probability of accidental death from the nuclear plant is smaller than from a similar fossil-fuelled plant.

The greatest weakness in this analysis is the estimate of health costs of air pollution. Ultimately, the most significant estimates will follow establishment of damage functions for each pollutant and the ability to evaluate total human impact of such exposures from known meteorological and chemical behaviour in the environment.

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Are BL Lac-type objects nearby black holes?

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Isolated black holes accreting interstellar gas can account for the salient properties of the BL Lac-type objects. The observed frequency spectra in the visible and infrared, the rapid variations in intensity and polarisation, and the absence of discrete features in the optical spectra are all consequences of the adopted black hole accretion model.

STRITTMATTER *et al.*¹ have discussed the possible existence of an entirely new class of astronomical objects with the following properties: (1) extremely rapid variations in the radio, infrared, and optical intensity; (2) most of the energy is emitted at infrared wavelengths; (3) absence of emission or absorption lines in their optical spectra; and (4) substantial and variable polarisation at optical and radio wavelengths. The prototype and most extensively studied object in this class is the variable star BL Lacertae (VRO42.22.01). Other Lacertids, as members of this class are now known, include OJ287 (VRO20.08.01), ON231 (W COM), ON325 (B2 1215+30) and PKS1514-24 (AP Lib).

Optical and near infrared spectrophotometry of the objects¹⁻⁴ reveal a steepening decline in the flux density from 10^{14} to 10^{15} Hz. The spectral shape, together with the observed rapid variations in intensity and polarisation, indicates that the observed radiation from the Lacertids results from nonthermal processes. The rapid variations further suggest that the emission originates from a very small region ($\leq 10^{17}$ cm). As a consequence of their nonthermal spectra, compactness and variability, these objects have been likened to quasistellar objects. But no redshifts can be measured because of the absence of spectral lines; so their distances remain unknown. Moreover, in the case of BL Lac, the very steep slope of the spectral-energy distribution above $10^{14.5}$ Hz suggests that this object is not a QSO (refs 3, 5).

As an alternative, we wish to point out that isolated black holes accreting interstellar gas can account for the characteristic properties of the Lacertids. The suggestion that these compact nonthermal sources may be massive ($\geq 10^4 M_\odot$) black holes has already been proposed by Pringle *et al.*⁶. Here we discuss the possibility that the BL Lac-type objects are nearby, stellar-mass black holes ($1M_\odot \leq M \leq 100M_\odot$) in the galactic disk.

Basic model

The total luminosity and frequency spectrum of radiation emitted by interstellar gas accreting on to a black hole have recently been calculated for spherically symmetric, steady state accretion^{7,8}. In this case, if there is a finely tangled and randomly oriented magnetic field in the interstellar gas, the rapid reconnection of oppositely directed radial field lines promotes an equipartition of magnetic and gravitational energy throughout the infalling plasma, ($B^2/8\pi \approx GM\rho/r$). Synchrotron radiation by relativistic electrons gyrating around the magnetic field lines is the principal radiation mechanism in the gas, resulting in a total observed luminosity

$$L_s \simeq 5 \times 10^{30} (T_0/10,000 \text{ K})^{-3} (M/10M_\odot)^3 (n_0/10 \text{ cm}^{-3})^2 \text{ erg s}^{-1} \quad (1)$$

provided $\beta = (T_0/10,000 \text{ K})^{-3/2} (M/10 M_\odot) (n_0/10 \text{ cm}^{-3}) \ll 10$. Here, n_0 and T_0 are the density and temperature, respectively, of the ambient gas and M is the mass of the black hole. If the black hole is moving supersonically through a uniform medium with a velocity V , the results of the calculations still apply provided we set $kT_0 \approx m_i V^2$, where k is Boltzmann's constant and m_i is the ion mass. The calculated spectral energy distribution falls sharply with frequency throughout the range $v_1 \leq v \leq v_2$, where

$$v_2 \simeq 7 \times 10^{15} (n_0/10 \text{ cm}^{-3})^{1/2} (T_0/10,000 \text{ K})^{-3/4} \text{ Hz} \quad (2)$$

is the peak synchrotron emission frequency in the region immediately outside of the Schwarzschild radius at $r = 2m = 2.95 \times 10^6 (M/10M_\odot) \text{ cm}$ and

$$v_1 \simeq 2 \times 10^{13} (n_0/10 \text{ cm}^{-3})^{40/49} (T_0/10,000 \text{ K})^{60/49} (M/10M_\odot)^{31/49} \text{ Hz} \quad (3)$$

is the characteristic frequency below which synchrotron radiation is self-absorbed by the gas.

Comparison with observations

The continuous emission spectrum is plotted in Fig. 1 for the accretion of ionised hydrogen with $T_0 = 10,000 \text{ K}$ (corresponding to $V \simeq 10 \text{ km s}^{-1}$) on to a single black hole. Emission spectra are shown for various interstellar gas densities and black hole masses and the results are compared with the data plotted by Strittmatter *et al.* for the BL Lac-type objects. Each theoretical curve has been fitted to the observed data for BL Lac at approximately $10 \mu\text{m}$. This corresponds to placing the black hole at a distance.

$$d \simeq 10 \text{ pc } (n_0/10 \text{ cm}^{-3}) (M/10M_\odot)^{3/2} \quad (4)$$

For all curves most of the energy is emitted in the infrared part of the spectrum in agreement with the observed spectra. The theoretical curve computed for $n_0 = 10 \text{ cm}^{-3}$ and $M \simeq 10 M_\odot$ is in reasonable agreement with the observed spectral energy distribution of BL Lac in the visual and infrared to $10 \mu\text{m}$.

Irregular time variations in intensity and polarisation are a natural consequence of an accretion model. Inhomogeneities in the ambient medium as well as instabilities and turbulence associated with the flow will inevitably lead to significant fluctuations in the total emission from the gas. These fluctuations are expected to occur over a variety of time scales. For example, intensity variations may result when the hole moves out of one turbulent cell of interstellar gas and into another⁹. The time scale for such variations is roughly

$$\Delta t_{\text{urb}} \simeq r_c/u_0 \simeq (10 \text{ yr}) (M/10M_\odot) (u_0/10 \text{ km s}^{-1})^{-3} \quad (5)$$

where $r_c = GM/u_0^2 \simeq 5 \times 10^{14} (M/10M_\odot) (u_0/10 \text{ km s}^{-1})^{-2} \text{ cm}$ is the capture radius of the black hole and $u_0 = (a_0^2 + V^2)^{1/2}$, where a_0 is the ambient sound velocity. On shorter time scales magnetic field reconnection, which is believed to be responsible for solar flares (see ref. 10 and references therein) may lead to strong flaring in the luminosity of the accreting gas. In the proposed accretion model the largest scale size associated with magnetic reconnection is roughly r^* , where r^* is the radius at which the frozen-in magnetic field has been sufficiently amplified by the compression of the infalling fluid that $B^*{}^2/8\pi \approx GM\rho^*/r^*$. In the zone $r_c \geq r \geq r^*$, lying just above the reconnecting region, the field is frozen in, requiring that $B \simeq B_0(r_c/r)^2$; so the reconnection rate at r^* , is given by

$$\Delta t_{\text{rec}} \simeq r^*/v_A \simeq (0.01 \Delta t_{\text{urb}}) (B_0/3 \times 10^{-6} \text{ gauss})^2 (n_0/10 \text{ cm}^{-3})^{-1} (u_0/10 \text{ km s}^{-1})^{-2} \quad (6)$$

where v_A is the Alfvén speed at r^* and B_0 is the ambient field strength. Magnetic reconnection will occur throughout the region $r^* \geq r \geq 2m$, maintaining an equipartition of magnetic and gravitational energy in the plasma. The longest timescale associated with magnetic flaring is $\Delta t_{\text{mag}} \approx \Delta t_{\text{rec}}^*$ or

$$\Delta t_{\text{mag}} \simeq (0.1 \text{ yr}) (B_0/3 \times 10^{-6} \text{ gauss})^2 (n_0/10 \text{ cm}^{-3})^{-1} (M/10M_\odot) (u_0/10 \text{ km s}^{-1})^{-5} \quad (7)$$

A sudden release of magnetic field energy and magnetic pressure should accompany each flare, causing an enhanced flow of plasma toward the hole and a corresponding burst of luminosity lasting a period Δt_{mag} . Finally, the shortest timescale for luminosity fluctuations is presumably associated with the infall rate ($\simeq$ reconnection rate) near the Schwarzschild radius,

$$\Delta t_{\text{min}} \simeq 2GM/c^3 \simeq (10^{-4} \text{ s}) (M/10M_\odot) \quad (8)$$

The random outbursts lasting ~ 1 month which occur on BL Lac and OJ 287 (refs 11–14, 18) agree with the predicted duration of a magnetic flare, assuming stellar-mass black holes and typical interstellar conditions (see equation (7)). BL Lac also exhibits more gradual luminosity modulations lasting about 10 yr (ref. 12). This long period variability may be associated with inhomogeneities in the interstellar medium in accordance with equation (5). Similar optical variations, occurring on a timescale of years, have been observed in OJ 287 (refs 14, 15). Short period variability ($\ll 1$ d) has also been observed for these two objects^{3,14–17}, as would be expected from an accretion model.

A net polarisation of the emitted synchrotron radiation would not occur in the case of spherically symmetric flow and finely tangled and randomly oriented fields. But inhomogeneities in the ambient medium, angular momentum of the gas, instabilities and turbulence may all lead to a highly variable, random net polarisation. The polarisation variations would then be correlated with the flaring activity of the source, a result consistent with the observations^{5,18}.

As shown by the dotted lines of Fig. 1, the synchrotron radiation is strongly self absorbed below $\nu \sim \nu_1$; so another radiation mechanism must be invoked to explain the radio emission from the objects. In the infalling plasma, conditions are favourable for the generation of radio emission through

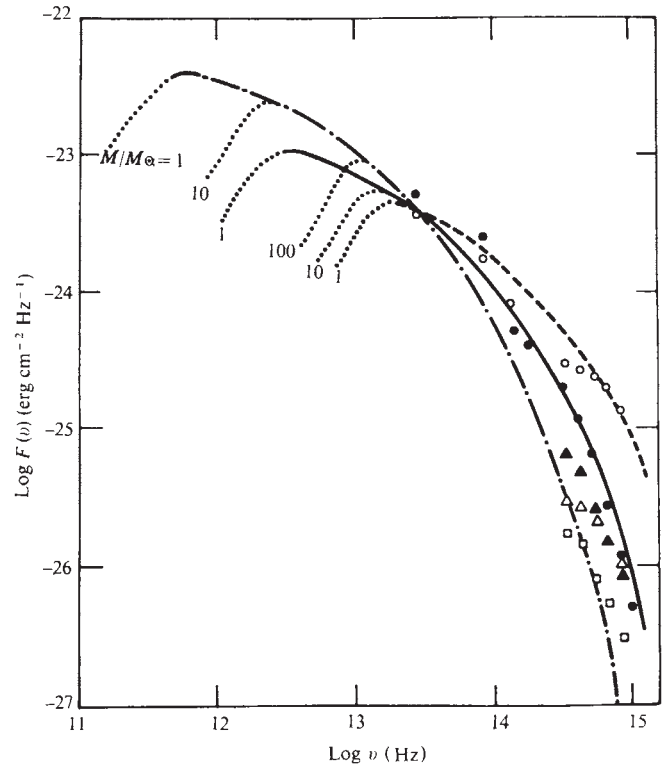


Fig. 1 The continuous emission spectrum from interstellar gas accreting on to a black hole compared with the observed spectra of the BL Lac-type objects as plotted in Strittmatter *et al.*¹. The gas consists of pure hydrogen with $T_0 = 10,000 \text{ K}$ ($V \approx 10 \text{ km s}^{-1}$); each curve is labelled by the corresponding value of black hole mass M . The dotted lines indicate synchrotron self-absorption. ●, BL Lac; ○, OJ 287; □, ON 231; △, B2 1215+30; ▲, AP Lib. —, $n_0 = 1 \text{ cm}^{-3}$; —, $n_0 = 10 \text{ cm}^{-3}$; —, $n_0 = 100 \text{ cm}^{-3}$.

coherent processes. Because of steep density and temperature gradients, longitudinal plasma waves and transverse electromagnetic waves will be coupled in the plasma. The possibility then exists for converting some of the electrostatic energy contained in plasma oscillations into radiation, a process which has been proposed as the origin of the enhanced radio emission from active regions on the Sun^{19,20}. The emission from a particular region is confined to the narrow frequency band $\nu_p < \nu < 1.4 \nu_p$, where $\nu_p = (e^2 n / \pi m_p)^{1/2}$ is the plasma frequency. In the model, the radio emission in a given frequency band centred about ν originates from the thin spherical shell of gas at r in which $\nu \simeq \nu(r)$, where

$$\nu_p(r) \simeq 2 \times 10^{10} (2M/r)^{3/4} (n_0/10 \text{ cm}^{-3})^{1/2} (u_0/10 \text{ km s}^{-1})^{-3/2} \text{ Hz} \quad (9)$$

If the emission is proportional to the available flux of gravitational potential energy passing across each shell ($L \propto GM\dot{m}/r$, where $\dot{m}$ is the accretion rate in gm s^{-1}) then the radio emission spectrum will vary as

$$L_\nu \propto \nu^{1/3}, \nu \leq \nu_p(2m) \quad (10)$$

in rough agreement with observations below 10^{11} Hz (ref. 1). For each of the BL Lac-type objects the radio emission is only a small fraction of the total luminosity. In the case of BL Lac, $L_{\text{radio}}/L_{\text{IR}} < 0.04$. Since the proposed synchrotron emission process itself converts only a small fraction of the available gravitational energy into radiation ($\sim 0.01 \beta$), the conversion of gravitational energy into radiation by plasma oscillations can be quite inefficient and still produce the required flux.

Predictions

Model calculations⁷ predict that, in addition to infrared and optical radiation, a high energy tail of γ -ray radiation between 1 and 10 MeV will be emitted due to electron-proton and electron-electron bremsstrahlung in the plasma. The total bremsstrahlung luminosity is

$$L_B \approx 2 \times 10^{26} (T_0/10,000 \text{ K})^{-3} (M/10M_\odot)^3 (n_0/10 \text{ cm}^{-3})^2 \text{ erg s}^{-1} \quad (11)$$

Unfortunately, for typical interstellar conditions and stellar-mass black holes, this γ -ray flux is far below the detectability limit of modern devices, even for nearby objects.

Rough estimates indicate that there may indeed be a finite number of stellar-mass black holes close to the Earth as required by the theory. From the known stellar birth rate function in the disk, stellar evolution time scales, and numerical computations of supernova explosions, it has been estimated⁹ that as much as 1% of the mass of the Galaxy may now be in the form of black holes with masses $\geq 10M_\odot$. This implies a local space density of black holes

$$n_{\text{bh}} \sim 2 \times 10^{-3} \text{ pc}^{-3}, \quad (12)$$

indicating that there could be eight such objects within 10 pc of the Earth.

A proper evaluation of the proposed model is not possible without a reliable determination of the distances to the objects. According to Pigg and Cohen²¹, the 21-cm absorption profile of BL Lac suggests that the object is at least 200 pc away and may be extragalactic. This lower limit would be substantially overestimated if a dense interstellar cloud is associated with BL Lac. If BL Lac and the other such objects are located within a few tens of parsecs of the Earth, their proper motions could be detected by both optical and radio techniques.

If it is determined that the BL Lac-type objects do indeed lie outside of the galactic disk, a black hole accretion model may still apply if any of the following are true:

(1) The infalling material possesses sufficient angular momentum to form an accretion disk about the black hole. In this case, the conversion of gravitational energy into infrared radiation *via* synchrotron emission may be far more efficient than in the case of spherical accretion, even for stellar-mass objects and typical interstellar conditions.^{6,22}

(2) The Lacertids are clusters or loose associations of stellar-mass black holes.

(3) The Lacertids are supermassive black holes situated in the galactic halo or at greater distances⁶.

These three possibilities clearly require further theoretical investigation.

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Restoration of specific immunological virginity

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The specific abrogation of an established immune response was studied by removing in vivo most of the immunocompetent lymphocytes reactive against the specific antigen, thus restoring the animal to a state of specific immunological virginity.

THE specific abrogation of the allergic response *in vivo* has been described in various experimental systems. In the induction of immunological tolerance^{1,2}, there is a central failure of the allergic response either as a result of clonal elimination or inactivation of specific antigen-sensitive cells. The existence of serum blocking factors which prevent the expression of an established allergic response has recently been described^{3,4}. In this case antigen-specific lymphocytes exist *in vivo* but fail to express their specific reactivity on account of serum blocking factors thought to be immune complexes⁵.

Here we describe another approach to the specific abrogation of an established immune response. This has been done by removing *in vivo* most of the immunocompetent lymphocytes reactive against a given antigen thereby restoring a primed animal to a state of specific immunological virginity.

Model system

The system makes use of a model^{6,7} involving cannulation of the efferent lymphatic from a single lymph node in sheep and subsequent drainage of lymph from the cannulated node. In this model it has been shown⁷ that macromolecules and lymphocytes which enter the parenchyma of a lymph node, leave the node only by way of the efferent lymphatic and cannot re-enter the peripheral blood directly from the lymph node.

We wondered whether there is an antigen-specific, selective mechanism in stimulated lymph nodes which enhances the entry into the node of lymphocytes specific for the antigen. If this is so, then by draining the efferent lymph from an antigen stimulated node, it should be possible to specifically abrogate the response of a primed animal, restoring it to a state of specific immunological virginity.

Random bred ewes from 1-4 yr old were injected with 2.5 human doses of BCG (Bacillus Calmette Guerin) and then primed to fluorodinitrobenzene (FDNB) by skin painting both ears with 100 μ l of FDNB in a 1:1 solution of acetone and olive oil. The animals were injected 10 d later with 10 mg of rabbit gamma globulin (RGG) and finally with 10 mg of NIP chicken globulin (NIP-CG). The injection schedule was phased to avoid antigenic competition⁸ and all injections were

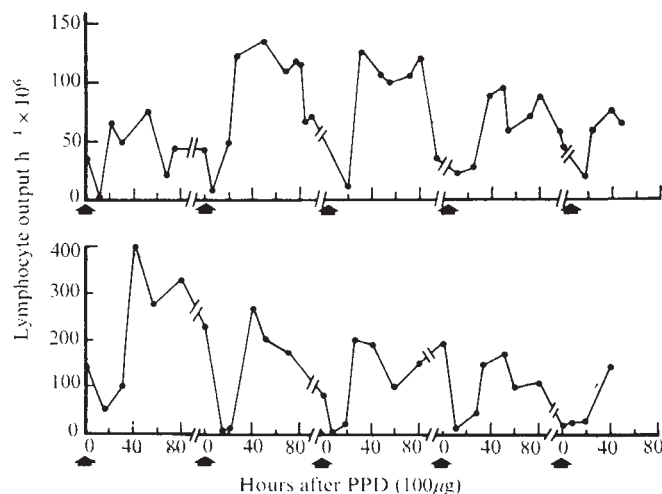


Fig. 1 Kinetics of lymphocyte output in the efferent lymph of BCG sensitive sheep after repeated stimulation of the cannulated lymph node with PPD. Heavy arrows indicate injection of 100 µg PPD in saline.

given intramuscularly in the shoulder region. The hapten NIP (4-hydroxyl-5-iodo-3-nitrophenacetyl-) was conjugated to CG as described⁹, the resulting molar ratio being 15 NIP groups per CG molecule. Both RGG and NIP-CG were given as alum precipitates together with 4×10^9 heat killed *B. pertussis*. The sheep were thus primed to two haptens and several protein carriers.

The popliteal lymph node was then cannulated and both lymph and lymphocytes collected twice a day for 3–4 weeks. During this time PPD (purified protein derivative of tuberculin, Batch 288, Central Veterinary Laboratories, Weybridge, Surrey) was injected repeatedly into the drainage area of the node. The second local challenge with PPD was given as NIP-PPD (mean conjugation ratio 0.5 NIP groups per PPD molecule) and the anti-NIP response measured in both efferent lymph and serum. This assays for PPD-specific 'helper' T cells.

Response

After 3–4 weeks cannulation was stopped and the systemic T cell reactivity of the whole animal to PPD and other antigens was measured both by skin testing and the 'helper' T cell assay using hapten-carrier conjugates. Antibody to the hapten (NIP) is formed only if there are helper T cells present which recognise the carrier, PPD or CG (ref. 10). The hapten-binding

capacity (ABC) of the serum or lymph was measured by the Farr Assay¹¹ using 10^{-8} M N^{125} IP caproic acid as hapten¹². The antigen binding capacity was calculated from the linear portion of the binding curve between 3% binding (1 unit of antibody ml^{-1}) and 30% binding (10 units of antibody ml^{-1}). Sera or lymphs were serially diluted such that two dilutions lay between 3 and 30% binding.

The kinetics of lymphocyte output in the efferent lymph of two cannulated sheep are shown in Fig. 1. In each case local challenge of the node with PPD produced a typical response in lymphocyte output characterised by a period of cell shutdown lasting 20 h followed by a large increase which is nearly always biphasic. The magnitude of the response decreased with repeated challenge.

During this response the second injection of PPD was given as NIP-PPD and the anti-NIP responses measured in both efferent lymph and blood. The first two sheep (Fig. 2) showed a brisk response to the hapten in the efferent lymph of the stimulated node. There was, however, no concurrent antibody response in the serum of these sheep, indicating that neither antigen nor antibody reaches the general circulation from the stimulated node. Thus PPD-specific T cells are present in the cannulated sheep at this stage and cooperate with hapten-specific B cells to initiate antibody formation to NIP.

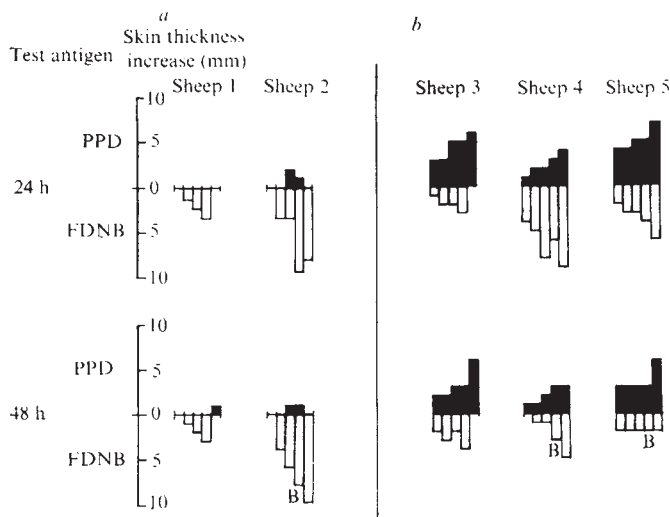


Fig. 3 Delayed hypersensitivity skin reactions to intradermal challenge with PPD and FDNB in cannulated (a) and control (b) sheep. PPD used at 6, 12, 25, 50 and 100 µg. FDNB (100 µl) used at concentrations 0.1, 0.2, 0.5, 1 and 2%; B, biopsy.

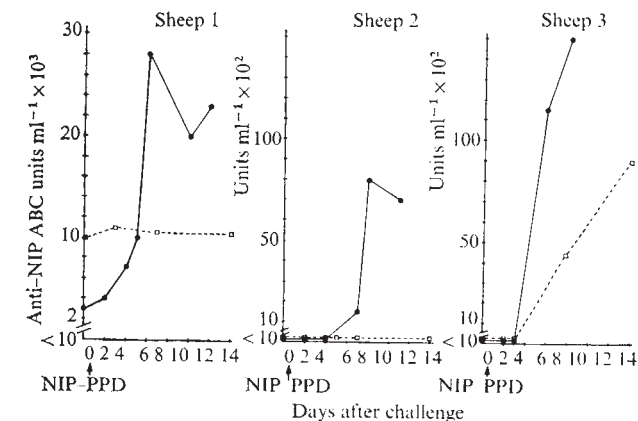


Fig. 2 Challenge of cannulated lymph nodes with NIP-PPD. Anti-NIP response in blood and efferent lymph following local challenge of the cannulated lymph nodes with NIP-PPD. Antigen binding capacity (ABC) in serum or lymph expressed as units of antibody ml^{-1} of neat serum or lymph. ●, Lymph ABC; □, serum ABC.

In the third sheep an anti-NIP response was detected in the serum as well as in the lymph. This cannulation stopped spontaneously after 8 d so it is likely that either collateral efferent lymphatics or lymphatic-venous connections had developed, affording systemic antigen escape and stimulation.

After five injections each of 100 µg PPD into the drainage area of the popliteal lymph node, the cannulations were stopped and the sheep tested systemically for delayed hypersensitivity skin reactions to PPD and FDNB by intradermal challenge on the flank. In contrast to the control sheep (Fig. 3) the cannulated sheep were virtually skin-test negative at 24 and 48 h after intradermal challenge to concentrations of PPD ranging from 6 to 100 µg. Two of the control sheep (3 and 4) were challenged five times at approximately 100 h intervals with 100 µg of PPD into the drainage area of the right popliteal lymph node, before intradermal challenge. Other control sheep were not injected with PPD before intradermal challenge and produced in each case similar reactions to PPD (sheep 5).

To check the specificity of this unresponsiveness the same sheep were similarly skin tested with FDNB (Fig. 3). In each

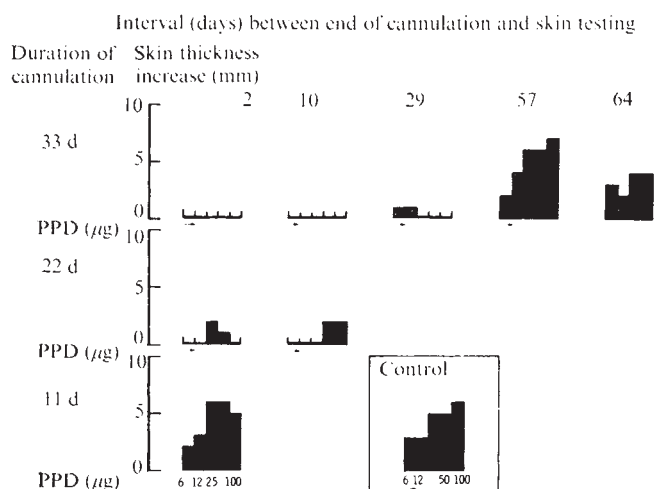


Fig. 4 Delayed hypersensitivity skin reactions to PPD in sheep cannulated for intervals of 33, 22 and 11 d and tested at several intervals after cannulation had stopped. PPD concentration as for Fig. 3.

case 100 μ l of FDNB at concentrations ranging from 0.1–2% in 4:1, acetone:olive oil was painted on the left flank. As shown in Fig. 3, there was little difference between cannulated and control sheep with respect to their delayed reactions measured at 24, 48 and 62 h after challenge. In non-FDNB primed sheep (sheep 5), challenge with FDNB produced a marked inflammatory response with skin thickening which is largely due to oedema and infiltration of the site with polymorphonuclear leukocytes. This nonspecific reaction, can obscure the specific response particularly at 24 h, but by 48 and even 62 h later the inflammatory response decays whereas in primed sheep the delayed reaction persists. Skin biopsies of the reaction to 1% FDNB in primed sheep showed typical mononuclear infiltration and histologically both cannulated and control sheep were indistinguishable. Although the cannulated sheep were not skin tested with PPD before the start of cannulation it was noted that the first few injections of PPD below the lymph node produced a strong delayed reaction in the tissues surrounding the injection site.

The delayed hypersensitivity reactions to PPD of sheep cannulated for various days and then tested at frequent intervals after cannulation had stopped are shown in Fig. 4. It seems that sheep have to be cannulated for at least 21 d before they

become specifically unresponsive to the antigen used. A sheep cannulated for only 11 d and injected three times with PPD showed little diminution in its delayed reaction to PPD. The reappearance of PPD reactivity in the sheep cannulated for 33 d is presumably due to the fact that BCG vaccination is a living infection and antigen persisting within the macrophages of the cannulated sheep stimulates virgin T lymphocytes as they emerge from the thymus.

Delayed hypersensitivity skin reactions are only one measure of T cell function. Attempts were also made to determine whether this specific unresponsiveness was also reflected at the level of specific 'helper' T cells, by challenging the sheep

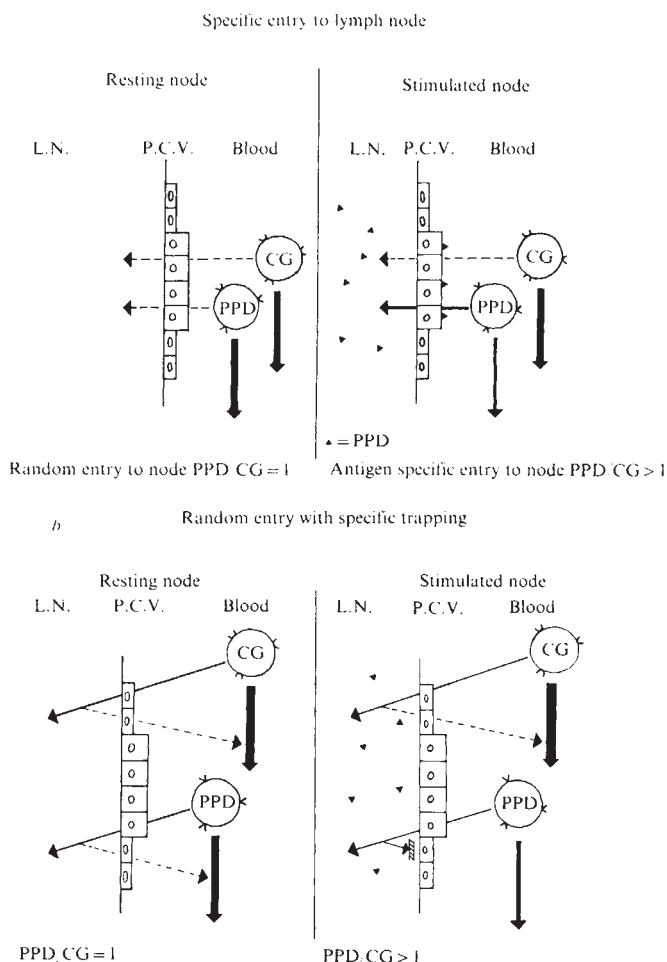


Fig. 6 Hypothetical models to explain specific depletion of antigen-reactive lymphocytes from the whole animal. *a*, Specific entry to lymph nodes; *b*, random entry with specific trapping. Explanation in text. PPD, PPD-reactive lymphocytes. CG, CG-reactive lymphocytes. LN, Lymph node. PCV, Post capillary venule. PPD/CG, ratio of specific cell types in lymph node.

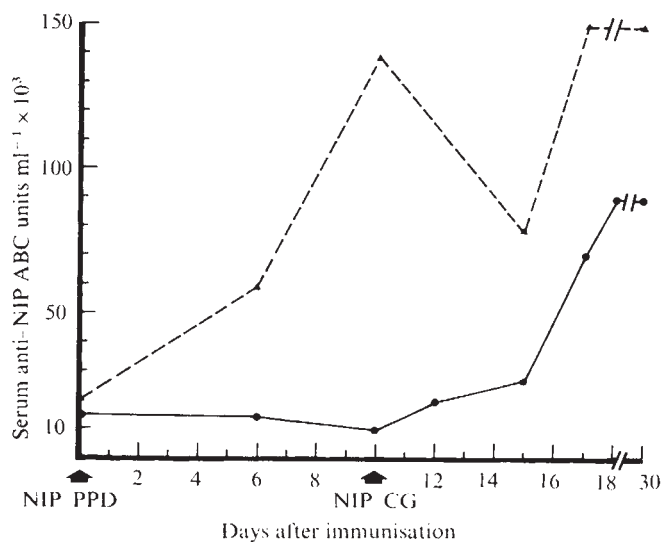


Fig. 5 Serum antihapten response in cannulated and control sheep after intravenous challenge with NIP-PPD and then NIP-CG. Serum ABC are expressed as units of antibody ml^{-1} neat serum. ●, Cannulated; ▲, control.

with NIP-PPD at a time when they were still unresponsive to PPD. This has been done in two sheep with the same result, the typical response in one of them being shown in Fig. 5. Compared with the control, cannulated sheep fail to make any serum anti-NIP response on intravenous challenge with NIP-PPD. This unresponsiveness of 'helper' T cells was also found to be specific since challenge with NIP-CG 10 d later when the animals are still unresponsive to PPD (see Fig. 4) does result in an anti-NIP response, which was approximately 70% of that seen in control sheep.

Using these two criteria of specific T cell function, the results indicate that cannulation of the efferent lymphatic from a single lymph node of primed sheep, together with repeated stimulation of the cannulated node results in specific abrogation of the allergic response to the stimulating antigen. This

phenomenon is similar to that in which the lymphocytes involved in the mixed lymphocyte reaction can be specifically depleted from the total recirculating pool when allogeneic lymphocytes are injected into a cannulated lymph node (ref. 13 and R. S. Cahill, H. Frost, and Z. Trnka, in preparation).

Removal of antigen-reactive lymphocytes?

Our explanation for this phenomenon is that there is an antigen specific selective mechanism operating at the level of stimulated lymph nodes which specifically removes antigen-reactive lymphocytes from the total recirculating pool. Since T cells are predominantly a mobile population of cells the net result of this mechanism is to specifically deplete antigen-reactive T lymphocytes from the entire animal.

Other explanations based on some form of tolerance induction² or specific desensitisation¹⁴ would demand that either antigen or blocking factors entered the systemic circulation from the lymph node. Failure to produce a serum antibody response following local challenge of the node with NIP-PPD whilst sustaining an anti-NIP response in the efferent lymph is evidence that neither antigen nor antibody reaches the systemic circulation from the lymph node and exits only through the cannulated efferent lymphatic. In two similarly injected control animals the skin reactions to PPD in one were slightly weaker than noninjected controls but there was no difference in their anti-hapten response after challenge with NIP-PPD. This indicates a slight degree of desensitisation in such animals with regard to delayed hypersensitivity skin reactions, but this is not reflected at the level of 'helper' T lymphocytes.

At least two mechanisms can be proposed to explain antigen specific selection (Fig. 6). Assuming that the proportion of PPD-reactive and CG-reactive lymphocytes are the same in peripheral blood (PPD/CG=1) then if lymphocytes only crossed the high endothelial cells of the post-capillary venule (PCV) randomly this ratio would remain the same and systemic depletion, when it did occur, would be non-specific. To explain specific depletion it could be argued that over and above random entry into stimulated lymph nodes there is a specific entry mediated by antigen attached to the luminal surface of the high endothelial cells of the PCV which preferentially selects PPD-reactive lymphocytes into the node (Fig. 6a). The net result is that within the node the ratio of PPD-specific: CG-specific lymphocytes is greater than 1 and specific depletion from the peripheral blood gradually occurs. It seems likely that at each passage through the lymph node only a fraction of the specific cells would be removed at any one time.

There is a precedent for membrane-bound antigen attached to the surface of macrophages¹⁵ in lymph nodes but not as yet for endothelial cells. Since there is ample functional evidence to show that antigen never escapes systemically from a stimulated node^{7,16} such a model would demand that the antigen once bound to the surface of the high endothelial cell was rarely released into the circulation.

The alternative explanation is one which challenges the accepted fact that lymphocytes only move from blood to lymph and never in the reverse direction¹⁷. In this model (Fig. 6b) a proportion of the cells which enter the node then travel in the reverse direction from lymph node to blood. In stimulated nodes, antigen within the node specifically blocks the return of PPD reactive lymphocytes but not of CG-specific cells and again the net result in time, is a specific, systemic depletion of PPD reactive lymphocytes. The specific trapping of lymphocytes by antigen and their subsequent disappearance from the thoracic duct lymph has been described in other systems¹⁸⁻²⁰.

Our results at this stage do not enable a choice to be made regarding mechanisms but they do raise some interesting questions concerning the physiology of lymphocyte traffic (or circulation).

Finally, we point out that the experimental system described here affords a simple surgical means of specifically abrogating the allergic response of a primed animal to a given antigen. It remains to be seen whether this approach can be exploited in organ transplantation or in the specific abrogation of autoallergic disease.

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Some new concepts in immunological phylogeny

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Immunocompetence is first evident at the invertebrate level in coelenterates. Primordial cell-mediated immunity revealed by specific allograft reactions with at least short term memory is demonstrable in advanced invertebrates, whereas integrated cell-mediated and humoral immunity evolved progressively with the vertebrates.

SPECIFIC immunological competence with long lived memory is found in all vertebrate immune systems, associated with lymphoid cells, a thymus equivalent and the ability to produce serum antibodies. Two major pathways determining cellular (T lymphocyte) and humoral (B lymphocyte) immunity can be distinguished in all classes of vertebrates. Though most primitive among vertebrates, hagfish and lampreys (Cyclostomes) reject skin allografts

with concomitant development of specific immunological memory and produce IgM-type antibodies to potent xenogeneic antigens¹. Given such impressive capacity at this level of phylogeny, one must look to the invertebrates for the origins and early mechanisms of immunocompetence. Until recently, little was known about the molecular defence mechanisms of invertebrates beyond nonspecific phagocytosis. We now know that rudimentary immune responsiveness with at least short term memory exists among certain advanced invertebrates. At still lower levels of phylogeny, immunorecognition and immunoincompatibility reactions with impressive specificity are demonstrable, but a memory component is doubtful². In spite of information gaps, a new conception of immunoevolution is emerging: essential cellular (T-cell) immunocompetence evolved progressively among metazoan invertebrates long before the additional (B-cell) vertebrate capacity to produce immunoglobulin antibodies.

The best studied and highest level of immunoevolution characteristic of all vertebrates can be designated 'integrated cell-mediated and humoral antibody immunity'. The cytoarchitecture of the lymphoid system and the molecular diversity of antibodies produced attain progressively higher levels of complexity in the evolution from fishes to mammals²⁻⁴. Circulating immunoglobulin antibodies of one or more molecular classes are inducible in vertebrates (B-cell pathways) in addition to immunorecognition and cell-mediated immunity reactions (T-cell pathways) also evident in invertebrates². Elaborate homeostatic regulation, including still poorly understood T and B-cell cooperation and suppression, is operative in birds and mammals. This can be regarded as the 'Rolls-Royce model' of immunoresponsiveness with numerous interdependent pathways (Fig. 1).

Important details of immunoregulation, especially tolerance, immunological blocking, memory and mechanisms of specific cell-mediated immunity, remain controversial or unknown. To pursue the Rolls-Royce analogy, a less elaborate Land Rover model of essential immunocompetence is found among fishes and amphibians, while bicycle models now appear characteristic of invertebrates. Depending on the environmental circumstances of course, the bicycle or Land Rover may be superior to the Rolls-Royce. One looks then for adaptive specialisation of the defence mechanisms among distinctive groups which have survival value or assure the integrity of the body. Among vertebrates, progressive diversification in molecular classes and subclasses of immunoglobulins is evident in the transitions from primitive fishes to mammals^{4,5}.

The ancestral immunoglobulin gene probably coded for a polypeptide of 110-112 amino acids corresponding to the

separate constant and variable region domains of antibody molecules⁶. By gene duplication and diversification of heavy and light polypeptide chains presumably guided by adaptive selection, the IgM immunoglobulin found in all vertebrates had already evolved in Cyclostomes several hundred million years ago. Why this complex antibody protein should be primitive is still puzzling, although its polymeric structure and multiple antigen-binding sites obviously increase the probability of combination with any antigen that has entered the body. Even at this level, further adaptive specialisation is apparent: IgM is tetrameric in bony fishes, hexameric in anuran amphibians and pentameric in certain sharks and mammals. Moreover, both lymphocyte and granulocyte-series of immunocytes are found in advanced invertebrates and all vertebrates, but the cytoarchitecture of the lymphoid system varies greatly at different levels of phylogeny⁷. Clearly different groups of animals have evolved different immunological equipment to meet their needs.

Primordial cell-mediated immunity

The capacity to develop specific transplantation immunity with concomitant immunological memory has been demonstrated in two phyla of advanced invertebrates—annelid worms^{8,9} and echinoderms^{2,10}. Echinoderms are more immediately ancestral to the vertebrates whereas annelids represent a line of evolution which diverged early from the progression leading to vertebrates. Slow but specific rejection of orthotopic integumentary allografts has been found in several species of earthworms, sea cucumbers and sea stars. The earthworm story is complicated by the unexplained intrapopulation compatibility *versus* incompatibility between geographically separate populations. However, integumentary xenografts between earthworm species are invariably rejected after a compatible healing-in period of considerable duration. At least short term immunological memory is evident in earthworms as revealed by accelerated rejection of repeat grafts placed at the time of or soon after initial rejection. However, the memory situation is more complex as indicated by prolonged survival of repeat grafts placed some time after initial allograft or xenograft rejection. Apparently, memory can be either short or long lived and positive or negative in its effect on foreign tissue survival in worms. This is an intriguing problem of specific immunoregulation. The observed transplantation immunity in worms is inherent in populations of leukocytes (coelomocytes) as shown by adoptive transfer experiments. Passive transfer of immune serum is without effect and immunoglobulins have not been detected in annelids.

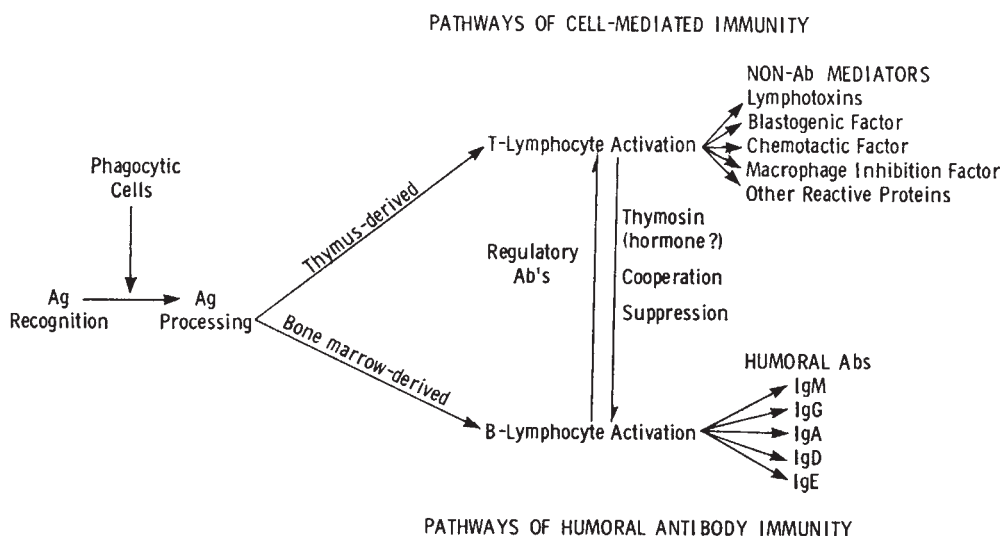


Fig. 1 Integrated pathways of cell-mediated and humoral antibody immunity at the mammalian level of immunoevolution. Elaborate and as yet poorly understood regulatory mechanisms are involved.

Echinoderms, even more than annelids, possess a rich assortment of leukocyte types, including distinctive lymphocytes, granulocytes and macrophages. Thus, mammal-oriented immunologists should not be surprised at the mounting evidence of immunological competence in the form of primordial cell-mediated immunity (PCMI) in these advanced invertebrates. Specific allograft immunity with at least short term memory was found in a sea cucumber (*Cucumaria tricolor*)¹⁰. Similar allogeneic incompatibility has now been found in two sea stars, *Protoreaster nodosus* from Australia¹⁰ and *Dermasterias imbricata* from California (Karp and Hildemann, unpublished). Leukocytes of multiple types, including macrophages, small lymphocytes (haemocytes) and eosinophilic granulocytes, were observed to infiltrate echinoderm allografts. Although the histological picture and chronic inflammatory reactions accompanying allograft rejection in these species are similar to those seen in vertebrates, the memory component may be distinctive. Experiments now in progress in our laboratory to compare the kinetics and consequences of short term compared with long term memory could be decisive in defining possible limitations of PCMI.

The lore of cell-mediated immunity (CMI) in mammals has expanded rapidly in recent years. The responsible cells are thought to be thymus-derived or T lymphocytes which originate in bone marrow. However, macrophages, granulocytes or equivalent cells cooperate critically with T lymphocytes, especially in the killing of foreign target cells. Moreover, T lymphocytes have a long life span and constitute the predominant small lymphocyte in the circulation as shown by their distinctive antigenic markers. Also T cells are the reservoir of long term immunological memory and are generally required as so-called helper T cells for the activation of antibody-producing B lymphocytes. Mammalian T cells are also distinguished by their characteristic responses to mitogens, such as phytohaemagglutinin (PHA), concanavalin A and *E. coli* lipopolysaccharide, reactivity in allogeneic mixed lymphocyte culture (MLC) reactions, and ability to mediate allograft reactions or delayed hypersensitivity reactions. The extent to which these properties of T cells persist as such throughout phylogeny remains to be determined.

Karp, Reddy, Tam and I (unpublished work) are variously investigating T cell type responsiveness in an echinoderm (*Dermasterias*), a tunicate (*Ciona*), a hagfish (*Eptatretus*) and a shark (*Heterodontis*) which together represent a transition group between the advanced invertebrates and lower vertebrates. Although all show similar patterns of chronic allograft rejection even at elevated water temperatures as evidence of PCMI, not even the hagfish and sharks seem capable of serum alloantibody responses. Blood leukocytes from these four species in culture respond distinctively to certain mitogens. Specific cell-surface recognition units or ancestral immunoglobulin receptors could be detectable on echinoderm or protochordate lymphocytes and pertinent results should be forthcoming. In short, although we are a long way from understanding cellular immune functions in phylogenetic perspective, the fact of cell-mediated immunity in advanced invertebrates is unassailable¹. Such CMI is sharply discriminating at the level of antigen recognition, but could be primordial in lacking diversification of effector functions or a long term memory component.

Immunorecognition and immunoincompatibility

Allogeneic transplants in ciliate protozoans and free living amoebae remain viable, but intergenus or xenogeneic transplants have always resulted in eventual cell death¹. No immunological component is evident in the incompatible combinations. Interspecies incompatibilities in sponge cell aggregation seem to depend on binding affinities of cell-surface glycoproteins rather than any immunological

surveillance system¹¹. Species-specific adherence of sponge cells is analogous to tissue-specific cell aggregation in higher animals. No cell damage or killing has been reported as a result of incompatible sponge cell reactions, although this possibility deserves closer inspection. However, immunorecognition or capacity for non-self recognition of allogeneic tissue followed by incompatibility reactions seems characteristic of the Coelenterata or Cnidaria. Indeed, allogeneic incompatibility is now well documented in colonial hydroids¹², gorgonians¹³ and hard corals¹⁴. Straightforward enzyme-substrate or biochemical incompatibilities are not responsible, because allografts and even xenografts heal compatibly for days or weeks before tissue destruction occurs. I would now designate this 'immunorecognition/immunoincompatibility' because of the apparent specificity of the antagonistic reactions and the compatible lag period preceding manifestations of incompatibility.

The experimental evidence concerning allogeneic incompatibilities in coelenterates detailed in the references cited above can be summarised briefly. Allogeneic colonies of the hydroid *Hydractinia echinata* fail to fuse when grown in contact, whereas colonies derived asexually from single colonies merge compatibly. Hyperplastic growth occurs in areas of direct contact between two incompatible colonies and regression of the overgrown colony usually ensues. A hierarchy of hyperplastic potential was found among diverse strains of *Hydractinia* and breeding experiments revealed complex genetic control of histocompatibility¹². Both allografts and xenografts in gorgonian corals are subject to rejection, but control autografts survive indefinitely. Ectodermal as well as ectodermal-mesogial grafts are reported to behave similarly. Incompatibility reactions were scored as greater with xenografts (*Eunicella stricta* $\rightleftharpoons$ *Lophogorgia sarmentosa*) than with allografts¹³. Surprisingly, disintegration of foreign transplants began after 4–5 d, even at the low temperature of 10°–15° C. Tests of specificity of rejection or immune memory have not been reported.

Staghorn corals of *Acropora* species are also amenable to decisive tissue transplantation experiments¹⁴. Branches from the same colony or clone fuse compatibly after 6–7 d in contact at 25° C and persist indefinitely with no sign of antagonism. Intercolony allografts generally show moderate incompatibility leading to soft tissue (coenenchyme) destruction in the contact zone, several to many weeks after initial functional fusion. Probable xenografts between *Acropora* species of uncertain taxonomic status yield detectable soft tissue death at the interface as early as a week after contact is made. Functional graft survival is easily tested by focal probing of polyp tentacles, which induces a withdrawal response instantly transmitted across a viable graft-recipient interface through the nerve net. Compatible coral grafts exhibit intact polyps and persistence of pigmented zooanthellae in the coenenchyme in zones of contact just as found in normal tissue. To what extent sharp immunological specificity or possible memory is involved in chronic rejection of allografts and subacute rejection of xenografts among these various coelenterates remains to be ascertained.

Tunicates of the protochordate group are generally held to be immediately ancestral to the vertebrates, thus being advanced invertebrates far removed from the coelenterates. As might be expected, tunicates have differentiated leukocytic cells including abundant lymphocytes. Immunoincompatibility or transplantation immunity at the level of colony specificity is common in colonial tunicates such as *Botryllus* species¹⁵, *Perophora viridis*¹⁶ and *Amaroeicum* species¹⁷. The tunic and vascular stolon of compatible colonies placed in contact fuse to form a continuous tube with exchange of blood cells. With histoincompatible colonies the tunic at the site of contact thickens without fusion and local necrosis

may subsequently occur similar to the allogeneic reactions characteristic of coelenterates. Our tests for allogeneic immunoincompatibility in the solitary tunicate, *Ciona intestinalis*, show capacity to recognise and reject orthotopic allografts of integument in contrast to persistent survival of control autografts (Reddy, Bryan and Hildemann, unpublished). *Ciona* lymphocytes also respond to PHA in a manner similar to mammalian T lymphocytes. Tests with other mitogens and of MLC reactivity are underway. Existence of PCMI with memory as found in echinoderms remains to be shown in tunicates, but the precedent capacity for immunorecognition/immunoincompatibility is surely well developed. The latter capacity then may be predicted for all invertebrates amenable to testing.

MLC-type reactions in mammals, and, one would predict, other vertebrate classes, display essentially the same properties of inducible incompatibility that are found in coelenterates and tunicates. Positive reactions between allogeneic lymphocytes are reflected in new DNA synthesis and appearance of blast cells suggestive of the hyperplastic contact reactions seen in coelenterates. In mice, rats and humans, the capacity of lymphocytes to respond in MLC can be separated from the CMI capacity to kill foreign cells or reject allografts^{18,19}. Since the MLC reaction requires the two populations of cells to be alive and antigenically disparate, active immunorecognition is involved. Preimmunisation is unnecessary and a memory component is either not involved or has a short term effect on the degree of MLC responsiveness²⁰. The whole range of antigenic disparities from strong to weak can evoke positive MLC reactions¹⁹, although strong histocompatibility antigens yield the highest MLC responses in mammals²⁰. So MLC-type reactivity probably represents an ancient immunosurveillance mechanism which has persisted in phylogeny since the appearance of eumetazoan invertebrates. At lower levels of invertebrate phylogeny, immunorecognition precedes the occurrence of lymphoid cells as such, but the recognition function could be inherent in surface receptors of epithelial-type cells²¹. Allogeneic stimulation of the MLC-type is not a cytotoxic reaction. Subsequent generation of cells or molecules able to mediate the killing of foreign cells in close proximity, however, usually follows *in vivo*²².

Conflicting interpretations

All the evidence, taken together, suggests certain major sequential steps in the phylogeny of immunological reactivity (Fig. 2). The first level of cell-specific or species-specific aggregation is fundamental to all colonial or multicellular organisms. Epithelial or other cell surface proteins govern compatible tissue formation in plants, protozoans and sponges, but these, often exquisitely specific, reactions lack an immunological character in the strict sense that incompatible cell killing or heightened responsiveness are not inducible. Specific immunorecognition

tion/immunoincompatibility is first evident at the invertebrate level of coelenterates, while cell-mediated immunity with at least short term memory has been detected initially in advanced invertebrates, notably annelids and echinoderms. There are obviously many information gaps in terms of invertebrate phyla not yet studied decisively or at all. I suggest that immunoincompatibility as regularly shown by allogeneic contact reactions is primitive and has persisted as an effective surveillance mechanism throughout metazoan phylogeny. MLC reactivity in modern birds and mammals is essentially similar to the allogeneic proliferative antagonism seen in coelenterates and tunicates. One may assume that progressively more differentiated leukocytic cells came to subserve this second level function as adaptive specialisation continued during phylogeny. Primordial cell-mediated immunity with memory constitutes a third level associated with cooperation of granulocytes and T lymphocytes in allograft rejection. This function becomes well developed in primitive fishes (cyclostomes and elasmobranchs) associated with longer-lived memory.

Integrated cell and humoral antibody immunity can be regarded as a fourth level development (Fig. 2) peculiar to vertebrates and perhaps restricted to advanced bony fishes and higher vertebrates. At this level helper T and B cells able to produce two or more molecular classes of antibodies are demonstrable. The cooperation of thymus-dependent lymphocytes (thymic hormone?) and certain B cells seems essential in mammals for the switch-over from specific IgM to IgG production²³. IgM production to quite potent immunogens may not require T and B-cell cooperation. Primitive fishes are capable only of IgM production to various antigens. Complex immunoregulation in birds and mammals (level 5) involves multiple molecular classes and subclasses of immunoglobulins in ways just beginning to be understood. The immunoregulatory mechanisms of primitive fishes making only IgM and of invertebrates with neither immunoglobulins nor a thymus gland are unknown.

Some immunologists are reluctant to accept the view that any adaptive immunity exists below the level of primitive fishes. Burnet²⁴ has considered that incompatibility in colonial tunicates and coelenterates as well as self-incompatibility in flowering plants are examples of self *versus* not-self recognition which are not analogous to the immunological processes of vertebrates. Similarly, Dausset *et al.*²⁵ regard the invertebrate incompatibilities as well as the MLC reaction in mammals as a non-immunological recognition system because a specific memory component is weak or absent. Other immunologists, however, view the allogeneic MLC reaction as an immunocompetent function and not simply the proliferative response of antigen-sensitive cells to contact with foreign cell membrane antigen^{20,22,26}. The point is that allogeneic stimulation may lead to the generation of cytotoxic lymphocytes with specific reactivity. The conflict

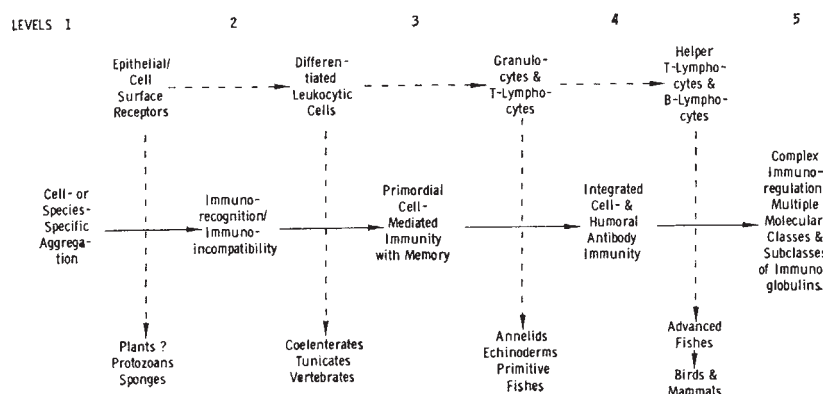


Fig. 2 Major steps in phylogeny of immunological reactivity. The suggested pathway is based on much new experimental evidence accumulated in recent years. Specific immunocompetence is already evident at the level of coelenterates, while cell-mediated immunity with memory is first detected in advanced invertebrates. Earlier manifestations of immunoreactivity seem to have been retained during progressive evolution and diversification of immunocyte functions.

is only partly one of definitions.

Extensive studies cited here indicate that immunorecognition/immunoincompatibility in coelenterates and tunicates involves not only non-self recognition of allogeneic tissue after initially compatible fusion; but also subsequent antagonistic reactions leading to localised cell death. In my view this constitutes the essence of specific, sharply discriminating, immune reactivity. Immunological memory associated with lymphoid cells may indeed be absent at the coelenterate level, but addition of these adaptive ingredients is clearly evident in echinoderms and probably also in tunicates. Available evidence suggests that cell-mediated immunity (T-cell function) evolved in invertebrates long before the additional vertebrate capacity for humoral antibody production (B-cell function). Among the vertebrates, progressive diversification of immunocyte functions appears correlated with increasing interdependence of the T and B-cell pathways. The supposition that immunological capacity paralleled leukocyte differentiation and evolved from no memory to short term to finally long term memory is attractive but unproved. Although the long known recognition system of nonspecific phagocytosis associated with the pioneering studies of Metchnikoff²⁷ is ubiquitous in metazoan phylogeny, granulocytes and other types of macrophages now appear to be critical participants in specific immune pathways and especially in cell-mediated immunity.

In essence, five levels of recognition and reaction to foreignness (Fig. 2) are now discernible in phylogenetic progression. All effective in maintaining the integrity of the organism and the species. If one demands both specificity and memory as qualifications for true immunological reactivity, then immunocompetence first appeared among the advanced invertebrates. But if the specific memory requirement is viewed flexibly also as an evolving characteristic, then immunocompetence is already manifest at the lower invertebrate level of coelenterates.

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Three-dimensional structure of adenyl kinase

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A combination of X-ray data at 3 Å resolution and sequence results has yielded the atomic structure of adenyl kinase, a ubiquitous enzyme which catalyses the phosphorylation of AMP by ATP. The abundant secondary structures of the protein and the probable binding sites for ATP and AMP are described.

ADENYL kinase (adenylate kinase, EC 2.7.4.3) catalyses the reaction¹ $\text{ATP} + \text{AMP} \xrightleftharpoons{\text{Mg}^{2+}} 2 \text{ADP}$. This interconversion of the adenine nucleotides seems to be of particular importance in organelles which have a high turnover of chemical energy, for example in chloroplasts², mitochondria³, or myofibrils⁴. With a molecular weight of 22,000 muscle adenyl kinase (myokinase) is the smallest of the known phosphoryl transferases⁵. Other kinases presently studied by X-ray

analysis have molecular weights of 40,000-100,000⁶⁻¹⁰.

A low resolution model of porcine muscle adenyl kinase¹¹ and its amino acid sequence¹² have been described recently. The X-ray analysis has now been pursued to higher resolution. A combination of 3 Å X-ray data and the sequence information yielded the atomic structure of the enzyme.

X-ray diffraction

It has been shown¹³ that porcine muscle adenyl kinase crystallises in one of the enantiomorphic space groups P3₁21 or P3₂21 with one molecule in the asymmetric unit. To find the correct alternative we made a separate measurement of 81 independent noncentric Friedel pairs with spacings above 10 Å (limit for negative Bragg angles on Siemens diffractometer) from the highly isomorphous CH₃HgNO₃ derivative. The observed structure factor amplitude difference within each Friedel pair was compared with the corresponding difference as calculated¹⁴ from the known protein structure factor¹¹ in conjunction with the known heavy atom structure factor¹¹. Correlation coefficients¹⁵ between observed and calculated differences were determined for both possible

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space groups. They turned out to be +0.80 and -0.80 for space groups $P3_121$ and $P3_221$, respectively, indicating that the true space group is $P3_121$. By chance, this space group had been selected when building the low resolution balsa wood model described earlier¹¹. Therefore, this model has the correct chirality.

The present structure analysis includes the 4,200 independent reflections that have spacings above 3 Å. For phase determination corresponding data sets were measured from the four heavy atom derivatives listed in Table 1. Reflection intensities were determined on an automated diffractometer following a procedure that has been described earlier¹¹.

Data were collected in shells of about 600 reflections each, that is, a complete data set contained seven shells. A separate measurement of about 500 reflections distributed over all shells was made for the protein and for every derivative. It overlapped with each shell in about 70 reflections. We used these overlaps to put the seven shells of a data set on a common scale. All data sets were then put on an absolute scale as derived from a Wilson plot¹⁸. This scale turned out to be 12% lower than the corresponding scale determined at low resolution¹¹.

All derivative data shells were corrected for systematic errors in the measurement before scaling and merging them to data sets. This correction was based on the assumption that the differences between derivative and protein structure factor amplitudes should be evenly distributed within a given shell. We checked for systematic deviations along the longitudinal and along the latitudinal coordinates on the surface of the shell. Such deviations were reduced by applying a correction factor to the derivative structure factor amplitude that was linear in both of these coordinates. The average correction on each amplitude was about $\pm 2\%$. This is not negligible because the phase analysis is based on the rather small differences between derivative and protein structure factor amplitudes¹⁶.

In the refinement of heavy atom parameters and in the Fourier synthesis we omitted those reflections that have intensities less than twice the estimated error of the measurement. This reduced the amount of data by 8%. In the refinement we followed a procedure given by Dickerson *et al.*¹⁷. It alternates between evaluation of the best phases and parameter shifts. The refined parameters are listed in Table 1.

The accuracy of the analysis as a function of resolution is illustrated in Fig. 1. Most of the phase information is derived from the CH_3HgNO_3 and the $\text{K}_2\text{Pt}(\text{SCN})_6$ derivatives because their heavy atom structure factors are much

higher than their lack of closure errors. The $\text{K}_2\text{Pt}(\text{NO}_2)_4$ derivative has a lower radial fall-off factor than the protein (see Table 1) and it is less radiation sensitive. Its contribution to the phase information, however, is rather poor. The lack of closure error exceeds the heavy atom structure factor at higher resolution. A second CH_3HgNO_3 derivative could be prepared by blocking the thiol groups asymmetrically with 6-thioinosine 5'-*o*-monophosphate (6-SH AMP) prior to soaking with CH_3HgNO_3 , so that effective substitution numbers changed (Table 1). As judged from Fig. 1 a considerable amount of phase information is obtained from this derivative. One has to bear in mind, however, that this derivative is not very different from the other mercury derivative. Therefore, their contributions to the phase information are to some extent coupled. As shown in Fig. 1 the figure of merit for phases drops 96% at low resolution to 77% at high resolution, the average being 82%. These values indicate that the electron density map is of reasonable quality.

Electron density map

The 'best' electron density distribution¹⁹ was calculated using the phased structure factors weighted with the corresponding figures of merit. The electron density map was computed in sections 0.94 Å ($=c/150$) apart perpendicular to the *c*-axis. These sections were plotted on microfilm and subsequently redrawn on perspex sheets at a scale 1 Å=4 mm. The resulting small map was very useful for chain tracing as well as for assigning side chains and preliminary α -positions.

With the amino acid sequence of the protein¹² at hand, chain tracing was not a great problem. All 22 markers: 14 aromatic side chains, six methionine and two cysteine residues could be found in the map and fitted. There is no density that has not been accounted for. Other chain tracings which become possible if one concedes large errors in electron density invariably lead to contradictions. Therefore, the fit of the amino acid sequence into the electron density map seems to be unambiguous.

A comparison of the resulting molecular boundaries with those determined at low resolution¹¹ showed no difference except that about five residues at the N-terminus had been incorrectly assigned to a neighbouring molecule, that is, they are at a wrong place (around residue 50, see Fig. 2) in the low resolution balsa wood model¹¹.

Table 1 Heavy atom parameters

Heavy atom compound	Soaking concentration (mM)	Soaking time (d)	R^*	$K^\dagger$	$\beta^\dagger$	$x^\ddagger$	$y^\ddagger$	$z^\ddagger$	$Z^\S$	$B^ $
CH_3HgNO_3	0.05	5	0.35	0.99	-2.9	0.480	0.857	0.115	70.0	8.0
						0.198	0.718	0.111	21.0	4.0
						0.503	0.737	0.121	12.0	22.0
$\text{K}_2\text{Pt}(\text{SCN})_6$	2.0	8	0.40	0.96	-4.8	0.565	0.711	0.111	90.0	11.0
						0.145	0.993	0.074	38.0	33.0
						0.221	0.977	0.103	27.0	28.0
						0.162	0.703	0.097	19.0	33.0
						0.336	0.149	0.044	7.0	45.0
$\text{K}_2\text{Pt}(\text{NO}_2)_4$	2.0	68	0.59	0.96	+1.3	0.546	0.698	0.109	40.0	11.0
						0.145	0.705	0.094	17.0	22.0
6-SH AMP and then CH_3HgNO_3	2.0	18	0.53	1.01	-2.9	0.480	0.858	0.115	38.0	1.0
						0.203	0.728	0.113	3.0	10.0

* Centric reliability factor¹⁸.

† Scaling parameters: Initially all data sets were scaled to equal ΣF at low resolution. In the refinement a scale factor $1/K \exp \beta S^2$ with $S = \sin \theta/\lambda$ was allowed for each derivative data set.

‡ Heavy atom positions in fractional coordinates.

§ Effective substitution number. Units are in numbers of electrons based on an absolute scale.

|| Radial fall-off factor¹⁷. The heavy atom structure factor is defined as $f_H = \sum_m Z_m \exp(-B_m S^2) \exp 2\pi i(hx_m + ky_m + lz_m)$ with the sum taken over all sites *m* of a derivative. The radial fall-off factor for the protein structure factor amplitudes is 9 Å².

The main sites of the $\text{K}_2\text{Pt}(\text{SCN})_6$ and the $\text{K}_2\text{Pt}(\text{NO}_2)_4$ derivatives as well as the third site of the CH_3HgNO_3 and the only site of a HAuCl_4 derivative¹¹ are located at the His-36 side chain in the cleft region. The first and second CH_3HgNO_3 sites are at Cys-25 and Cys-187, respectively.

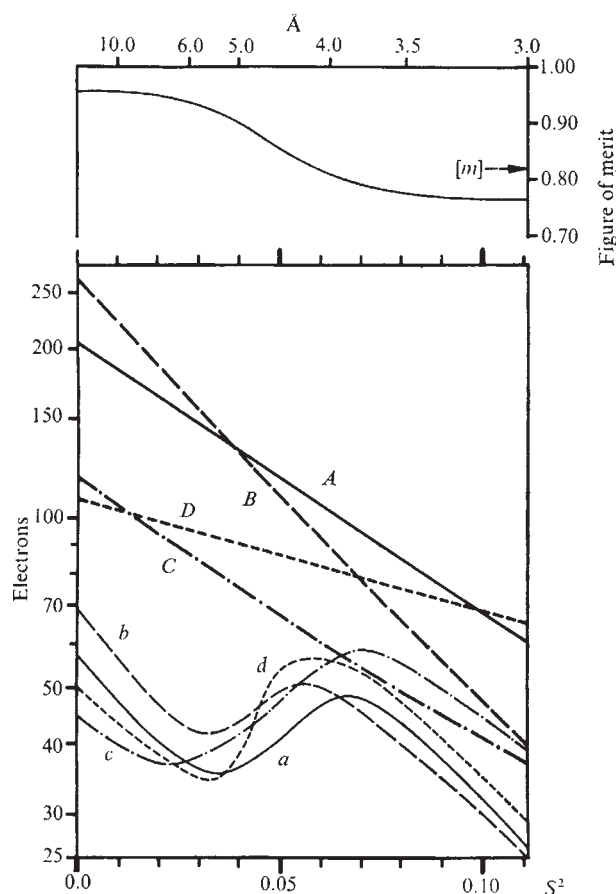


Fig. 1 Variation of root mean square (r.m.s.) heavy atom structure factors, r.m.s. lack of closure errors, and the figure of merit of the phases with resolution. Curves *A*, *B*, *C*, *D* are the r.m.s. heavy atom structure factors and curves *a*, *b*, *c*, *d* are the corresponding r.m.s. lack of closure errors for the derivatives: CH_3HgNO_3 , $\text{K}_2\text{Pt}(\text{SCN})_6$, $\text{K}_2\text{Pt}(\text{NO}_2)_4$, and $[\text{6-SH AMP} + \text{CH}_3\text{HgNO}_3]$, respectively. The plot is logarithmic with the resolution taken as $S^2 = (2 \sin \theta / \lambda)^2$. All r.m.s. lack of closure error curves have a trough at 5 Å corresponding to a similar minimum of the mean protein structure factor in this region. The overall mean figure of merit is 82%.

Description of the molecule

A stereo drawing of the polypeptide chain is given in Fig. 2. As with the low resolution balsa wood model¹¹ we selected the view that exposes the deep cleft. This cleft divides the molecule into two domains: The domain on the right side consists of three helical rods which enclose a

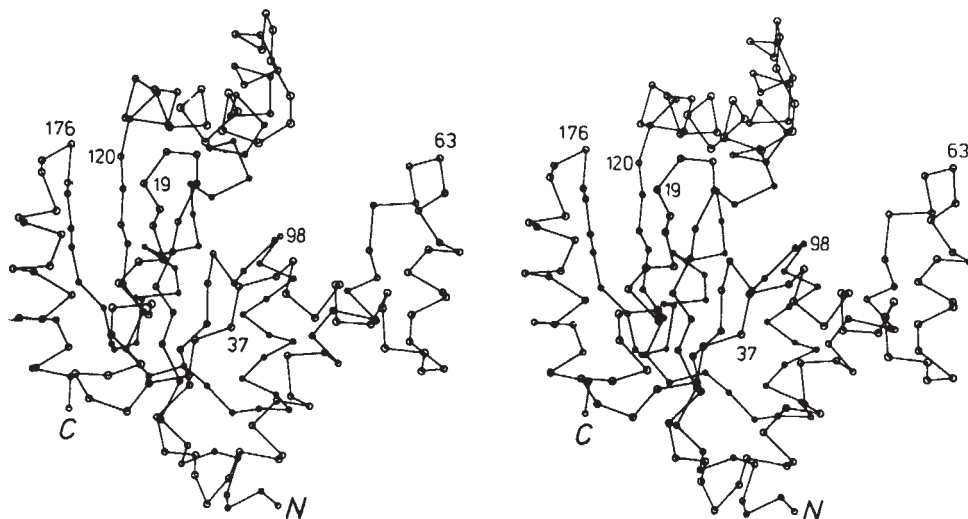


Fig. 2 Stereo drawing of polypeptide chain of adenyl kinase. C α -atom positions are marked with circles. N and C termini as well as some residue numbers are inserted. In the electron density map the chain fades out between residues 137 and 141 in an exposed region on the surface of the molecule. This stretch of chain, however, is strongly connected in the 6 Å map and in an intermediate 4 Å map.

small hydrophobic core. The domain on the left side of Fig. 2 contains a parallel pleated sheet formed by five strands, each about five residues long. It has a right-handed twist like the pleated sheets in many other proteins¹⁰. The sheet is the architectural centre of this domain. There are two hydrophobic cores, one on each side of the sheet. They are formed between the sheet and covering helices. About 55% of all residues are in right-handed helical configuration and 13% are in the sheet. Thus, about two thirds of all residues are involved in secondary structures. This probably accounts for the exceptional acid stability of adenyl kinase⁴. Several parts of the helices seem to be distorted from the α -helical configuration²¹. The relation between primary, secondary and tertiary structure is illustrated in Fig. 3.

All charged side chains are on the surface of the molecule. Being situated deep in the cleft aspartate-93 is an exception. There is an unusual piece of chain containing five glycines and one proline between residues 15 and 22 (Fig. 3). As shown in Fig. 2 it is situated in the cleft forming a crooked loop as expected from such a sequence²³. This loop envelops a dense narrow spherical peak that has double the height of the average backbone density. It is also higher and narrower than would be expected from a rigidly bound sulphate ion. Based upon height and width of the peak it seems to be due to a metal ion with an atomic number approximating those of Mn, Fe, Co, Ni, Cu, or Zn. Atomic absorption measurements on freshly prepared active adenyl kinase, however, revealed none of these metals (Gastner, personal communication). We suspect that the enzyme has picked up a metal ion during the preparation of the crystals; possibly this metal ion indicates the location of the Mg^{2+} or Mn^{2+} during catalysis^{24,25}.

Location of active centre

Adenyl kinase binds both AMP and ATP at specific sites¹. All attempts to bind substrates or substrate analogues to the crystals failed¹¹, and we must at this time rely upon indirect evidence to locate the active centre.

NMR data show that one of the two histidines in porcine muscle adenyl kinase is involved in catalysis²⁶. His-36 is located in the cleft region and binds many heavy atom compounds (see Table 1) whereas His-189 lies flat on a very smooth surface of the molecule. Carp muscle adenyl kinase contains only one histidine residue, and this is equivalent to His-36 in the porcine enzyme (von Zabern and Noda, personal communication). Since it is very likely that a residue involved in catalysis is conserved during species evolution²² we may assume that His-36 is at the active site, that is, that the active site is in the cleft region.

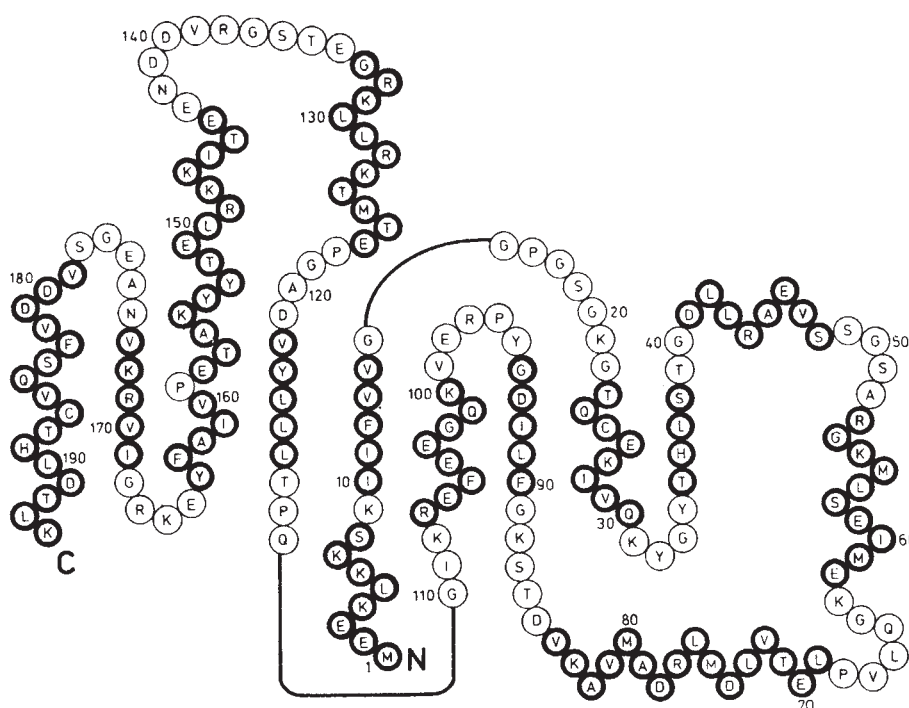


Fig. 3 Sequence of adenyl kinase in one letter notation²². Residues involved in helical structures and in the pleated sheet are drawn with heavier circles. The diagram indicates roughly the chain fold. The orientation of the molecule corresponds to that shown in Fig. 2. Helix 23-30 is above the plane of the paper and helices 100-107 and 144-164 are below.

The region around His-36 is rather conspicuous. Cys-25 is about 5 Å away. Such a close His-Cys-pair is probably also present in the active centres of creatine kinase²⁷ and of the Na-K-transport ATPase²⁸. Asp-93 is almost buried in the cleft. It may be hydrogen-bonded to His-36 and/or Cys-25. His-36, Asp-93, and Cys-25 are also present in the carp enzyme (von Zabern and Noda, personal communication). Ser-38 is very close to His-36 and Asp-93. The spatial relationship between these three residues, however, is that of an equilateral triangle and not that of a straight line as in the charge relay system of the proteases²⁹. The distances between the centre of the postulated metal ion and the centres of the side chains of His-36 and Asp-93 are 10 Å and 5 Å, respectively. There is sufficient space between His-36 and the postulated metal ion to accommodate a phosphoryl group. This group would also touch Asp-93. Moreover, there are several basic side chains in the cleft region. They might serve to neutralise the negative charges on the substrates.

There are two hydrophobic pockets nearby. One is formed in the region between Gly-20 and Val-179 (see Fig. 2), the other one between Gly-94 and Tyr-153, that is, in the cleft region. It seems geometrically possible to fill the first pocket with the adenine moiety of AMP and the second one with that of ATP in such a way that the phosphate group of AMP and the γ -phosphoryl group of ATP meet each other between His-36 and the postulated metal ion, that is, at Asp-93. This localisation of the substrates is comparable with the topology of the nucleotide binding sites in other proteins³⁰.

In the crystal the second pocket is partly covered by a neighbouring molecule. This might be one of the reasons why we have not succeeded in binding substrate to the crystalline enzyme so far. In future substrate binding studies we intend to concentrate on crystal forms of adenyl kinase¹¹ which have not yet been analysed.

We are indebted to K. Biedermann, G. Müller, and W. Sachsenheimer who contributed to this work; we thank Professor K. C. Holmes for encouragement and support.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Large flare on the red dwarf star UV Ceti

ATTEMPTS to measure radio emission occurring simultaneously with the sporadic optical flares on the red dwarf dMe type stars have been hindered by the rarity of large flares. Apart from one case of a flare with an unusually long duration and a peak magnitude of 1.7 (ref. 1) the correlation so far established has been limited to flares of about 1 mag or less^{2,3}. On October 11, 1972 we were able to obtain simultaneous radio and optical recordings of an unusually large flare on the star UV Ceti (L726-8AB), during which the apparent magnitude of the star increased by more than 4.55 mag.

The event occurred during the period of cooperative observations of this star⁴ (October 1-15, 1972). The observations described here were made simultaneously by the Mk 1A radio telescope at Jodrell Bank, and the 30-inch Cassegrain reflector ($f/13.5$) at the Stephanion Astronomical Station in Peloponnese. The radio telescope was working on a frequency of 408 MHz (± 2 MHz to 3 dB). The aerial received linear polarisation and the output was obtained by the digital sampling process¹. A radio source of known strength was used for calibration. A Johnson dual channel photoelectric photometer⁵ was used on the 30-inch reflector.

The raw data in the optical case consisted of the photoelectric intensity I_t (minus the sky background) during the flare, compared with I_o , the photoelectric intensity (minus the sky background) for the quiet star. The light variation is plotted in Fig. 1 as the variation in the b colour magnitude $\Delta m(b) = 2.5 \log I_t/I_o$. During the peak of the flare between 21 h 18 min 52.2 s and 21 h 18 min 53.4 s UT the recording pen was off scale. The other interruptions in this record are caused by measurements of the sky background or by checks of the position of the star in the field of view of the telescope. In the radio case the data are the digital printout (logger units) from the sampling made five times a second and printed at intervals of 30 s. The scale of flux units at 408 MHz is derived directly from the similar digital printout on the calibration source (3C33). The calibration was made between 20 h 27 min and 20 h 43 min UT.

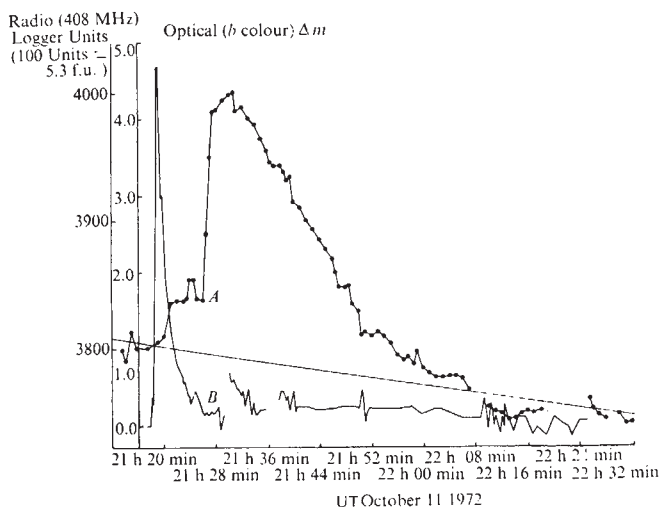


Fig. 1 The radio (A, 408 MHz) and photoelectric (B, b colour) records of the flare on UV Ceti on October 11, 1972. The ordinates for the radio record are digital logger units with 100 units = 5.3 flux units ($10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$).

No corrections have been made to these 408-MHz data plotted in Fig. 1 but because the flare occurred when the star was at an elevation of about 9° from Jodrell Bank there is a slight baseline slope caused either by ground effects or temperature drifts in the parametric amplifiers. The inclined abscissa indicates the estimated true baseline derived from recordings over the same azimuth-elevation range on preceding and following nights, when no flare occurred. In the case of the photoelectric records, measurements of a standard light source at 20 h 55 min and 23 h 06 min, differed by only 0.004 mag. Therefore, no corrections have been applied, and there are no corrections to $\Delta m(b)$ for atmospheric extinction. (The relative air-mass factors—secant of zenith distance—varied from 2.078 at 20 h 56 min, 1.777 at 22 h 44 min—upper culmination—to 1.787 at 23 h 06 min UT.) Measurements in the b colour of the companion star d (Fig. 1 of ref. 6) were made according to the recommendation of IAU Commission 27 (ref. 7). These measurements, at 20 h 56 min and 23 h 05 min UT were compared with the b colour measurements of UV Ceti at 21 h 06 min (quiescent state) and at 22 h 27 min UT. Flare star minus d were +0.54 mag and +0.45 mag respectively. The second order extinction coefficient K''_{b-v} at Stephanion is usually within 0.00 mag and -0.08 mag (ref. 8), and because $\Delta(b-v) \approx 1$ mag, the calculated values of these differences outside the Earth's atmosphere are approximately 0.62 mag and 0.52 mag respectively.

The rates of energy production during the flare are approximately $3.2 \times 10^{25} \text{ erg s}^{-1}$ over a bandwidth of 400 MHz in the radio spectrum, and $2.5 \times 10^{30} \text{ erg s}^{-1}$ over a bandwidth of $6.7 \times 10^8 \text{ MHz}$ in the optical spectrum. This ratio of 10^5 is similar to that for large solar flares but, as found in the previous measurements, the total energy output in the flare of 10^{31} to 10^{32} erg during the peak phase is at least equal to that of the normal photospheric continuum. In contrast, the total energy output of a large solar flare is equivalent to only about a millionth of the normal quiescent emission.

In many earlier radio-optical correlations there has been evidence that the peak of the radio emission follows the maximum phase of the optical flare by several minutes^{1,2}. The main feature of the correlation described here is the decisive evidence that the sharp front of the optical flare precedes that of the radio flare by approximately 8 min (the maxima are separated by 11 ± 1 min). The interpretation in terms of a solar flare type event in the atmosphere of UV Ceti is given in an accompanying paper⁹.

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Model for a flare on the star UV Ceti

ON October 11, 1972 a large flare occurred on the star UV Ceti. It was observed simultaneously at photoelectric frequencies and at the radio frequency of 408 MHz (ref. 1). These observations provide enough information to describe a reasonably good model of the outer layers of the star, and of the overall mechanics of the flare.

The radio flare must have occurred in a region with low gas density, otherwise the radiation could not have propagated away. According to the observations there was a delay of about 500 s between the onset of the flare at optical and at radio frequencies. During this interval the disturbance from the optical flare must have spread through the corona of the star. The inevitable conclusion is that UV Ceti has a corona and a stellar wind. The energy required to heat the corona and to drive the wind must, presumably, come from the mechanical energy of the convective motions in and near the photospheric layers.

The general properties of flare stars have been described². For the star UV Ceti the data are as follows: mass = 10^{32} g, radius = 4.5×10^9 cm, and therefore surface gravity $g = 3.3 \times 10^5$ cm s⁻². When conditions are quiescent the surface temperature is $T_* = 3,000$ K, and the flux of radiation is 4×10^9 erg cm⁻² s⁻¹. The star has a surface area of 2.5×10^{20} cm² and its luminosity is 1.0×10^{30} erg s⁻¹.

Opacity tables³ can be used to construct a rough model for the atmosphere of the star. The table for an "Iben Mixture XII", with $X=0.8$ and $Z=0.01$, seems most appropriate. It becomes immediately apparent that the atmosphere must be convective even considerably above the level at which optical depth $\tau = 2/3$ is reached. The outer layers of the star are therefore fully convective; the pressure P is found to be 3.6×10^7 dyne cm⁻² and the density is 1.7×10^{-4} g cm⁻³ at $\tau=2/3$. The table given by Cox and Stewart³ has to be extrapolated to reach these values of pressure and density; the formula $\kappa = 9 \times 10^{-39} \rho^{0.45} T^{11}$ fits the opacity data reasonably well in the region concerned.

To generate a radiant energy flux of 4×10^9 erg cm⁻² s⁻¹ requires that the photospheric layer which can effectively radiate should have a surface density of 16 g cm⁻². As $\rho = 1.7 \times 10^{-4}$ g cm⁻³ this layer is only $\approx 10^5$ cm thick. Outside this layer the gas can hardly radiate at all.

The scale height, H , in the photosphere is about 6.5×10^5 cm and I assume that the mixing length for the convective motion is the same. To produce a convective flux of 4×10^9 erg cm⁻² s⁻¹ the rising and falling currents need an average speed $U = 2.0 \times 10^4$ cm s⁻¹, and a rising (or falling) element must have a temperature excess (or defect) of $\Delta T = 7$ K with respect to the mean temperature.

It seems probable that the convective motions will twist up any magnetic lines of force above the star. The turbulent pressure in the photosphere is $\rho U^2 = 6.8 \times 10^4$ dyne cm⁻². If this is equated to the magnetic pressure $H^2/4\pi$, then an estimate $H = 900$ gauss is obtained for the likely magnetic field strength at the surface of the star.

The photoelectric magnitude of UV Ceti increased by more than $\Delta m = 4.5$ during the flare¹. The peak increase lasted about 10 s. At 4,500 Å (a frequency of 6.7×10^{14} Hz) the normal energy output of the star is

$$\pi R^2 (8\pi h \nu^3 / c^2) \exp(-h\nu/kT) = 1.6 \times 10^{14} \text{ erg Hz}^{-1} \text{ s}^{-1}$$

An increase of 4.5 mag raises the energy flux by a factor of 63, to about 1.0×10^{16} erg Hz⁻¹ s⁻¹. Over a bandwidth of, for instance, 6.7×10^{14} Hz this corresponds to an energy output of 6.7×10^{30} erg s⁻¹ during the flare. But the optical flare is presumably a localised phenomenon above the surface of the star, not equally visible from all sides. I shall assume that the flare of October 11 was on the side facing the Earth. The energy received from it at the Earth would then be above average. I allow a factor 2.5 or 3 to take account of this. The estimate for the rate of energy production by the flare then becomes 2.5×10^{30} erg s⁻¹. Over a period of 10 s the energy output is 2.5×10^{31} erg.

The flare must occur in the lower corona. I shall take the ambient gas density there to be ρ_0 and the magnetic field strength in the region of the flare at the time of its occurrence to be H_0 . In accordance with the usual interpretation of solar phenomena I shall assume that the energy of the flare is released during the reconnection of the lines of force in a magnetic field. The Alfvén speed in the region will be $V_A = H_0/(4\pi\rho_0)^{1/2}$; if L is the typical linear dimension of the flare region, the duration of the optical flare at its maximum can be estimated to be

$$\tau_0 = L/V_A = L\sqrt{(4\pi\rho_0)/H_0} = 10 \text{ s} \quad (1)$$

The total magnetic energy in the flare region is

$$\mathcal{E}_0 \approx H_0^2 L^3 / 8\pi \quad (2)$$

If the light produced by the flare is a result of synchrotron radiation by fast electrons, then for the critical frequency $\omega_0/2\pi$ to be about right requires that, at the time of the optical flare,

$$\omega_{c,0} \approx 4.2 \times 10^{15} \approx \gamma_0^2 e H/mc \quad (3)$$

where $\gamma_0 mc^2$ is the typical energy of the electrons. If there are $\mathcal{N}_0$ electrons altogether, their total energy is $\mathcal{N}_0 \gamma_0 mc^2$ and their rate of production of radiant energy is

$$Q_0 = \mathcal{N}_0 \gamma_0^2 e^4 H_0^2 / m^2 c^3 = \mathcal{N}_0 \omega_{c,0} (e^3 / mc^2) H_0 \quad (4)$$

The estimate for Q_0 is 2.5×10^{30} erg s⁻¹. It follows from equations (3) and (4) that

$$\mathcal{N}_0 H_0 = 6 \times 10^{36} \text{ gauss} \quad (5)$$

But from relation (3) $\gamma_0 = 1.6 \times 10^4 H_0^{-1/2}$ and so

$$\mathcal{N}_0 \gamma_0 mc^2 = 10^{35} H_0^{-3/2} \quad (6)$$

is the total energy of the relativistic electrons present at any one time during the optical flare.

Further information can be deduced from the events following the visual flare. I postulate that after the reconnection of the lines of force a bubble of plasma with a magnetic field expands into the stellar corona, preceded by a shock wave. About 500 s later the bubble reaches the level at which radiation at 408 MHz can just propagate through the corona. I shall take this level to be at a height h_1 above the base of the corona. The local density of the plasma can be found from the condition that its refractive index is 0 at 408 MHz and this leads to $\rho_1 = 5 \times 10^{-15}$ g cm⁻³.

The expansion of the bubble can be described if something is known about the variation of density with height in the stellar wind. I assume the wind to be isothermal, with a pressure-density relationship $P/\rho = a^2 = \text{constant}$. Rough estimates indicate that the sonic point in the stellar wind will lie one or two stellar radii above the photosphere. In such a wind the variation of speed, u , with height is given by

$$(u - a^2/u) du = (2a^2/r - GM/r^2) dr \quad (7)$$

(see for example Kahn⁴). At the sonic point

$$u = a \text{ and } r = r_s = GM/2a^2 \quad (8)$$

To find how u varies with r near the sonic point put $u = a(1 + \epsilon)$ and $r = r_s(1 + \eta)$. With these substitutions equation (7) may be solved, correct to the second order in ϵ and η , in the form

$$\epsilon = \pm \eta \text{ or } u - a = \pm(a/r_s)(r - r_s) \quad (9)$$

The upper sign here applies for the case of a stellar wind, and the solution then simplifies to

$$u = ar/r_s \quad (10)$$

I shall apply this approximation all the way from the base of the corona to the level at which the 408 MHz radiation

can propagate. Then conservation of mass in the wind gives constant $= \rho v r^2 = \rho a r^3 / r_s$ (11)

and the density at height h above the photosphere is

$$\rho = \rho_0 R^3 / (R+h)^3 \quad (12)$$

Magnetic energy $\mathcal{E}_0$ is released in the lower corona during the visual flare. After time t the bubble has grown to linear dimensions h from its initial linear size L . Conservation of magnetic flux demands that the field strength should vary as h^{-2} , so that at the later time the energy content is $\mathcal{E} = \mathcal{E}_0 L/h$, the energy density is $\mathcal{E}_0 L/h^4$ and the magnetic pressure $P = \mathcal{E}_0 L/3h^4$. To expand, the bubble must push back the ambient coronal gas. The condition of momentum balance at the boundary of the bubble means that

$$P = \rho V^2 = \rho \dot{h}^2 \quad (13)$$

where $V \equiv \dot{h}$ is the speed of expansion. It follows from equations (12) and (13) that

$$\rho_0 h^2 / (1+h/R)^3 = \mathcal{E}_0 L / 3h^4$$

and so the time τ_d required to reach height h_1 is given by

$$(\mathcal{E}_0 L / 3\rho_0)^{1/2} \tau_d = \int_0^{h_1} h^2 (1+h/R)^{-3/2} dh \quad (14)$$

The major contribution to the integral in this equation comes from the upper end of the range of integration, so that

$$\int_0^{h_1} h^2 (1+h/R)^{-3/2} dh \approx (2/3) h_1^{3/2} R^{3/2} \quad (15)$$

From equations (1) and (2) it follows that

$$(\mathcal{E}_0 / 3\rho_0)^{1/2} L^{1/2} = 6^{-1/2} (H_0^2 / 4\pi\rho_0)^{1/2} L^2 = 6^{-1/2} V_A L^2 = 6^{-1/2} L^3 \tau_0^{-1} \quad (16)$$

and so

$$6^{-1/2} L^3 \tau_d / \tau_0 = (2/3) h_1^{3/2} R^{3/2}$$

or

$$h_1 / R = (L^2 / R^2) (\tau_d \sqrt{3} / 2\tau_0 \sqrt{2})^{2/3} \quad (17)$$

For the flare of October 11, $\tau_d / \tau_0 = 50$, and the relationship becomes

$$h_1 / R = 9.8 L^2 / R^2 \quad (18)$$

Equations (1), (2), (12) and (18) together lead to a relationship between the energy $\mathcal{E}_0$ and linear dimensions L of the flare, in the form

$$\mathcal{E}_0 = (1/2) \rho_0 V_A^2 L^3 = \rho_0 L^5 / 2\tau_0^2 = (\rho_1 L^5 / 2\tau_0^2) [1 + (h_1 / R)]^3 = (\rho_1 L^5 / 2\tau_0^2) [1 + (9.8 L^2 / R^2)]^3 \quad (19)$$

Inserting values for $\rho_1 \tau_0$ and R leads to the estimate in Table 1.

Table 1 Typical values for the parameters

	10^9	2×10^9	3×10^9	cm
L	8.3×10^{28}	2.2×10^{31}	1.0×10^{33}	erg
$\mathcal{E}_0$	45	270	1,000	gauss
H_0				

The results (Table 1) can be approximately represented by the formulae

$$H_0 = 2 \times 10^{-21} L^{5/2}, \mathcal{E}_0 = 3 \times 10^{-48} L^{8.5} \quad (20)$$

Comparison of the results in equation (20) with that in equation (6) shows that the total energy of the relativistic electrons present in the flare at any one time is

$$\mathcal{E}_{rel} = \mathcal{N}_0 \gamma_0 mc^2 = 10^{66} L^{-15/4} \quad (21)$$

It is obvious that $\mathcal{E}_{rel} < \mathcal{E}_0$, because the electrons derive their energy from the magnetic field. A lower limit to the linear dimensions of the flare is therefore given by

$$L \geq 1.7 \times 10^9 \text{ cm} \quad (22)$$

When there is equality in equation (22), $\mathcal{E}_0$ equals 1.6×10^{31} erg, barely enough to account for the visual energy output of the flare. If $\mathcal{E}_0$ exceeds $\mathcal{E}_{rel}$, for instance $\mathcal{E}_0 = \alpha \mathcal{E}_{rel}$ with $\alpha > 1$, then

$$L = 1.7 \times 10^9 \alpha^{0.08} \text{ cm} \quad (23)$$

so that even with $\alpha = 100$, say, the estimate for L is raised by only a factor 1.5. Furthermore, because $\mathcal{E}_{rel}$ decreases as L increases, it seems most probable that $\mathcal{E}_{rel}$ can never be as large as the total visual energy output of the flare, and that the synchrotron lifetime of the electrons is shorter than the duration of the flare. It must therefore be possible for electrons to pick up energy from the magnetic field as the flare evolves.

In the light of all these arguments it seems reasonable to conclude that $L \sim 2.5 \times 10^9$ cm. This implies that $\mathcal{E}_0 = 3.0 \times 10^{32}$ erg, $H_0 = 600$ gauss, $\mathcal{E}_{rel} = 6.0 \times 10^{30}$ erg, $\gamma_0 = 640$ and

$\mathcal{N}_0 = 1.0 \times 10^{34}$. The height h_1 equals 1.4×10^{10} cm and the estimate for the density in the lower corona becomes $\rho_0 = 3.8 \times 10^{-13} \text{ g cm}^{-3}$.

By the time the magnetised bubble reaches height h_1 it has expanded by a factor 5.6 in linear dimensions, and the magnetic field strength has dropped, by a factor of 1/31, to 20 gauss. If the radiation at 408 MHz also results from synchrotron emission, the energy $\gamma_1 mc^2$ of the electrons is then given by

$$\omega_{c,1} \approx 2.5 \times 10^9 = \gamma_1^2 e H / mc = 3.4 \times 10^8 \gamma_1^2 \text{ or } \gamma_1 \sim 3$$

The observed output of the radio flare at its peak is 10 flux units (f.u.) $= 10^{-22} \text{ erg cm}^{-2} \text{ Hz}^{-1} \text{ s}^{-1}$. The distance to UV Ceti is 2.7 pc or 8×10^{16} cm and therefore the rate of output of energy by the star, per unit frequency interval, is $8 \times 10^{18} \text{ erg Hz}^{-1} \text{ s}^{-1}$, or about $3.2 \times 10^{25} \text{ erg s}^{-1}$, assuming a bandwidth of about 400 MHz. This means that there must be $\mathcal{N}_1$ electrons radiating, where

$$\mathcal{N}_1 \gamma_1^2 (e^4 H^2 / m^2 c^3) = (e^3 / mc^2) \mathcal{N}_1 H_1 \omega_{c,1} = 3.2 \times 10^{25}$$

There seem to be more relativistic electrons radiating during the radio phase of the flare than there were during the earlier, visual phase. But their individual energies are smaller, and their total energy at any one time is 4.0×10^{31} erg, about seven times that during the optical flare. For comparison, the magnetic energy in the bubble is now $\mathcal{E}_0 L/h_1 = 5.4 \times 10^{31}$ erg, still considerably larger than the particle energy. The acceleration process evidently still feeds energy to the electrons in the later stages of the flare.

The model of the flare has implications for the stellar wind. Take $r_s = 3R$ or $4R$ as possible values for the sonic radius. They correspond to

$$a = (GM/2r_s)^{1/2} = 1.7 \times 10^7 \text{ cm s}^{-1} \text{ or } 1.5 \times 10^7 \text{ cm s}^{-1}$$

and to temperatures $T = \bar{m} a^2 / k = 2.2 \times 10^6 \text{ K}$ or $1.6 \times 10^6 \text{ K}$. With the approximate form for the density variation in the stellar wind, the density at $r = r_s$ becomes $\rho_s = 1.4 \times 10^{-14} \text{ g cm}^{-3}$ or $6 \times 10^{-15} \text{ g cm}^{-3}$, and the predicted rate of mass loss

$$4\pi r_s^2 \rho_s a = 5 \times 10^{14} \text{ g s}^{-1} \text{ or } 3.4 \times 10^{14} \text{ g s}^{-1}$$

These values are not sensitive to the assumed value of r_s . They are of the same order as the mass loss rate inferred for another flare star, YZ CMi (ref. 4).

It is not obvious, with an isothermal model, how the amount of energy carried off by the stellar wind should be estimated, for in principle the wind speed keeps increasing indefinitely with distance. In practice, though, the isothermal condition is bound to fail at some reasonable distance. An acceptable first estimate is that each gram of stellar wind carries away energy of about $GM/R = 1.8 \times 10^{15} \text{ erg g}^{-1}$, and the total predicted energy loss into the wind is of the order of $8 \times 10^{29} \text{ erg s}^{-1}$. For comparison the luminosity of the quiescent star is $1.0 \times 10^{30} \text{ erg s}^{-1}$. The present model thus requires that a large fraction, at least 40%, of the energy brought by convection to the photosphere should later become available as mechanical energy for heating the stellar corona.

Finally, the magnetic energy stored in the region of the flare amounts to $\mathcal{E}_0 = 3.0 \times 10^{32}$ erg, localised above an area $L^2 \sim 6 \times 10^{18} \text{ cm}^2$ of the star. A supply of about $4 \times 10^9 \text{ erg cm}^{-2} \text{ s}^{-1}$ seems to be available as mechanical energy to drive the stellar wind. With an equal supply available to twist up the lines of force it would only take about $1.3 \times 10^4 \text{ s}$ (about 3.5 h) to charge up the magnetic field, ready for the flare.

I thank Sir Bernard Lovell for telling me about the observations of UV Ceti, and for discussion about its properties.

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Redshifts of 1548 + 115a and 1548 + 115b in the theory of the generalised gravitational potential

In a recent communication Wampler *et al.*¹ reported the redshifts of the QSO pair 1548 + 115a, b. For the QSO 1548 + 115a the redshift is given as $z = 0.4359$ and for 1548 + 115b as $z = 1.901$. Since these QSOs are separated by approximately 5 arc s these authors concluded, primarily on the basis of a statistical argument, that these two objects are at the same cosmological distance and that the redshifts are therefore "discordant". In addition these authors noted that the wavelength ratio $(1 + z)_b / (1 + z)_a = 2.02$, which is sufficiently close to a factor of two for them to speculate that for such optically associated objects this "... is either an unfortunate coincidence or a profound mystery".

Stellar objects that are apparently connected but have considerably different redshifts have been reported previously. Arp², for example, has reported on pairs of galaxies which are joined by luminous bridges but which have different galaxy redshifts, and Burbidge *et al.* (BBSS)³ have published a statistical analysis of the proximities of 47 QSOs to bright galaxies, concluding that four of these QSOs were probably associated with their respective galaxies. Again in the Burbidge work the redshifts for the pairs are "discordant."

The work of Arp and BBSS has been discussed previously^{4,5} within the context of the generalised gravitational potential which is inferred from my general relativistic scalar field theory in the complex Weyl space⁶. In this theory the macroscopic theories of Einstein gravitational and Maxwell electromagnetism are united together with the microscopic scalar field in Ψ . When this theory is applied to the "Schwarzschild" problem for the nucleus, where the Newton-Einstein constant is negligible, the integration constant in the component g_{00} of the metric tensor is identified with a nuclear property, namely the nuclear core radius. Therefore for a stellar object with a density comparable with that of nuclear matter, where both constants are significant

$$g_{00} = 1 - [(2GM/c^2) + r_0 A^{1/3}]/r \quad (1)$$

where G is the Newtonian gravitational constant, M the mass of the body, c the speed of light, r_0 the nucleon core radius and A the stellar mass number. The generalised gravitational redshift now becomes

$$z_G = [(2R_0 R/r_0 \rho^{1/3} (1 + R)) - 1]^{-1} \quad (2)$$

where

$$R = (r_0 c^2 / 2G m^{1/3}) M^{-2/3} \quad (2a)$$

and m is the nucleon mass, $R_0 = 1.21$ fm the nucleon radius and $\rho = D/D_0$ is the ratio of the density D of the stellar body to that of the density D_0 of the nucleon. Because of the "Schwarzschild" singularity we must also have in this theory that $z_G < 1$. And for a total redshift of z

$$z_v = (z - z_G / (1 + z_G)) \quad (3)$$

where z_v is the recessional redshift.

If we now apply the theory of the generalised gravitational potential to the pair 1548 + 115a, b and assume a negligible gravitational factor for 1158 + 115a (that is an object with both a negligible contribution from Einstein gravitation and a density much less than that of nuclear matter), we have $z_v = z = 0.4359$. If in addition we assume the maximum possible gravitational contribution from this theory ($z_G \approx 1$) we obtain from equation (3) $z_v \approx 0.451$ for 1548 + 115b; thus there is no need, in the context of this theory, to seek a further explanation of the wavelength factor of ~ 2 commented on by Wampler *et al.*

Stated in an alternative way, we assume that the total redshift given by Wampler *et al.* for 1548 + 115a represents the recessional redshift for both objects. In addition, however, we apply equation (3), utilising the maximum possible gravitational redshift from this theory ($z_G \approx 1$) for 1548 + 115b and obtain $z \approx 1.8718$ for 1548 + 115b, which is close to the result obtained by Wampler *et al.*

If the work⁷ of Cameron on neutron stars, which does not make use of equation (1), is nonetheless taken as a model for all stellar objects with neutron densities then the masses of these objects are of the order of one solar mass. It should be noted from equation (2) that gravitational redshifts in this theory can be appreciable for small masses. For example, for a solar mass with nuclear density ($\rho = 1$), $z_G = 0.776$. To account, therefore, for the energetics implied by the data on 1548 + 115b it has been suggested to me by Dr Howard Poss of Temple University that perhaps 1548 + 115b is actually an assemblage of such stars. This suggestion is, however, not to be restricted to the pair 1548 + 115a, b but must be generalised to any stellar system whose energetics cannot be sustained by a constraint of one solar mass, which is indeed how this general concept is applied in this theory for any stellar system with a "discordant" redshift. In addition, if cognisance is to be taken of the precision in the redshift calculations by Wampler *et al.* then one could postulate a small relative velocity between 1548 + 115a and 1548 + 115b with a resultant velocity component in the direction of the Earth for 1548 + 115a. This is a more definitive statement than that offered by these authors when discussing the precision of their measurements. Furthermore, if these authors mean by "spectral peculiarities" that there could exist associated QSO pairs with different redshifts not explainable on the basis of Einstein's theory and that therefore "... no pre-existing theory suggested them ...", then this statement is incorrect^{4,6}.

Both Bahcall *et al.* (BMB)⁸ and Hazard and Sanitt (HS)⁹ have pursued the work of BBSS by choosing different sample spaces. In the former case these authors considered a larger group of quasars in addition to the 3C quasars chosen by BBSS. Although they found additional quasars that are closer to bright galaxies than expected if these two types of objects were uncorrelated, the increase was not at all proportional to the BBSS finding. In this connection it should be noted that, from the point of view of the generalised gravitational potential, only three (3C232, 3C275.1, 3C309.1) of the original four in the BBSS list were consistent with a connection hypothesis, whereas the fourth (3C268.4) was not; that is to say that this theory predicts that observational data may be found to support the connection hypothesis for the first three quasars but no observational data will be found to establish a physical connection between 3C268.4 and NGC4138. If this theory were further taken into account then the original BBSS number would change from four to three probable associations. This in turn would modify somewhat the expectations of the work of BMB.

The latter work of HS also failed to confirm the BBSS results (restricted to 3CR types) when source and galaxy lists, other than those chosen by BBSS, were used. It should be noted that the elimination of 3C232 from the BBSS list would modify still further the expectations of BMB.

In an additional discussion Bahcall and Woltjer¹⁰ considered the pairs Ton 155, 156 and 1548 + 115a, b. They conclude that, when taken as a class, the close proximity of QSOs in each pair "may be comfortably explained as random coincidences ...".

The problem with all four papers quoted here^{3,8-10} that adopt a statistical approach to the redshift problem is that they each select (*a priori*) a subset of objects. The central physical problem, however, is whether or not there is an additional component in the redshift. The theory of the generalised gravitational potential claims there is, and to this end galaxy-galaxy pairs, QSO-galaxy pairs and QSO-QSO pairs should be examined with continually improved observational techniques, where the statistical analyses are of value only with respect to the choice of areas for concentrated observational effort. The question of whether or not QSOs are local or cosmological or consist of

both types of object will in any event obviously be influenced strongly by observational results obtained on the redshift problem.

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Infrared heterodyne spectroscopy of CO₂ on Mars

THERE has been considerable interest in applying infrared heterodyne techniques to astronomical problems¹⁻³. We have used an infrared heterodyne spectrometer operating near 11 μm with a resolving power of 1.5×10^6 to obtain spectral line profiles of carbon dioxide absorption in the Martian atmosphere. The multichannel system determined shapes of three lines in the 10^0 0-00° 1 vibration-rotation band of ¹³C¹⁶O₂, and found equivalent widths about 50 MHz (0.0017 cm^{-1}). A lower limit of 670 MHz (0.022 cm^{-1}) on the equivalent width of the P(20) line of ¹²C¹⁶O₂ was also obtained.

Figure 1 shows a block diagram of the equipment. Two concave mirrors not shown in Fig. 1 matched the diameter and wavefront curvature of the laser and telescope beams at the combining beam splitter. The 9% of the laser beam which was reflected by the beam splitter and the 91% of the telescopic beam which was transmitted were focused on to a copper-doped germanium photomixer cooled to 4 K. The photomixer signals were amplified over a frequency band from 100 to 1,500 MHz and fed into a radiofrequency mixer with a variable local oscillator frequency. The output of the mixer was fed into a filter bank which simultaneously measured the power in eight channels, each 18 MHz wide, equally spaced from d.c. to 150 MHz. Each channel had a synchronous detector locked to the chopping frequency and followed by a low pass filter. The eight outputs of the filter bank were sampled and digitised by computer. To cancel offsets between the two parts of the sky seen by the dual beam chopper, the planet was periodically alternated from one beam to the other.

As a periodic calibration of the system sensitivity, a black body at 500 K was placed in front of the chopper. By placing the heliostat in autocollimation, the telescope transmission was found to be 0.85. Atmospheric transmission was determined by measuring heterodyne signals from the Moon and Mars as a function of zenith angle. Averaged over the band of 100 to 1,000 MHz, the zenith transmission on the P(20) line of ¹²CO₂ (wavelength 10.57 μm) was 0.45, lower than may be expected from previous measurements⁴

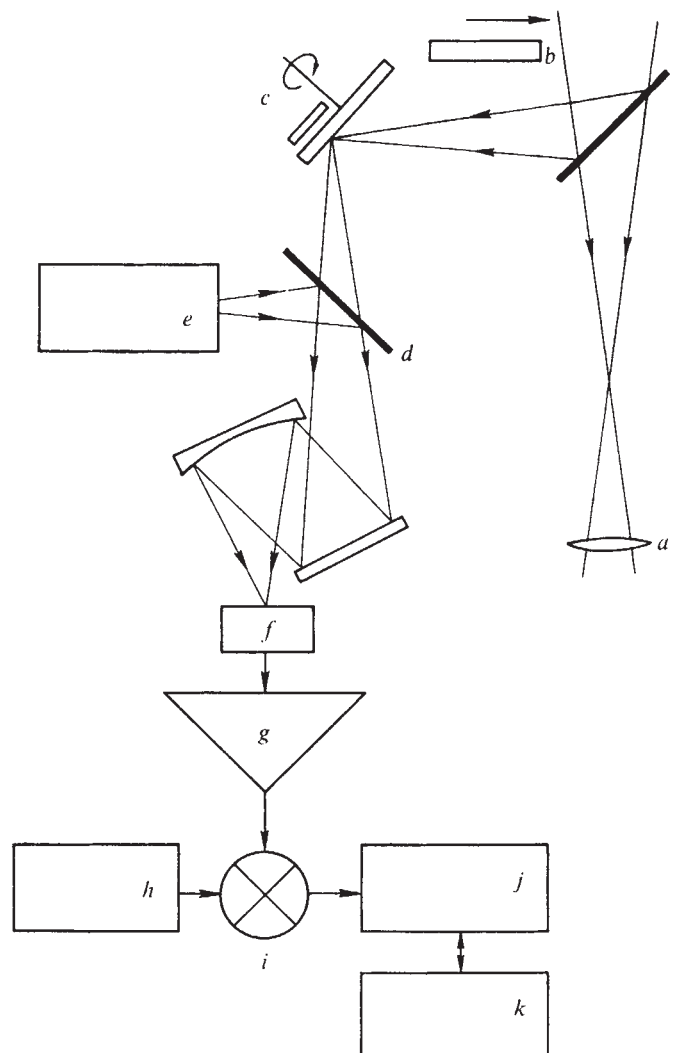


Fig. 1 Block diagram of the equipment located at the auxiliary solar telescope, located near the focal plane. *a*, Guiding eyepiece; *b*, black body; *c*, chopper; *d*, beamsplitter; *e*, CO₂ laser; *f*, germanium: copper photomixer; *g*, amplifier; *h*, radiofrequency local oscillator; *i*, radiofrequency mixer; *j*, eight channel filter bank; *k*, computer. The telescope consists of a steerable flat heliostat with a collecting area of about 0.4 m², followed by a fixed off-axis focusing mirror and three flat mirrors which direct the beam to the equipment. A rotating mirror in the focal plane of the telescope chopped the source against a sky background at 150 Hz. A linearly polarised laser used as an infrared local oscillator was operated in the TEM₀₀ mode, and stabilised on various rotational transitions of the 10⁰ 0-00° 1 band of CO₂.

and transmission of the ¹³CO₂ lines near 11.2 μm was 0.90. The approximate standard deviation in each case was 0.05. The contribution of water vapour to the absorption was estimated to be 0.05 to 0.10 (ref. 5). The system sensitivity for each 18 MHz channel was thus calculated to be 5×10^{-22} Wm⁻² Hz⁻¹ at the top of the Earth's atmosphere, assuming operation on a ¹³CO₂ line, radiation of one polarisation, and a signal to noise ratio of unity for one second of integration. Because of the antenna properties of the heterodyne receiver⁶ and the shape of the heliostat mirror, the system was only sensitive to radiation in an elliptical beam measuring 4 arc s in declination (dec) and 3 arc s in right ascension (RA). The angular diameter of Mars was about 14 arc s at the time of observation.

In order to understand how the spectral information was obtained it is necessary to consider how the spectrum was folded on itself in the two mixing operations (Fig. 2). The continuum infrared radiation is folded over into the absorption profile and decreases the fractional depth by a factor

of two. We assumed that the absorption lines were symmetric and tuned our radio frequency local oscillator to the expected centre of the absorption line. This frequency was calculated from the relative velocities and rotation rates of Earth and Mars, and typically had a value near 1,100 MHz. The output of the radio frequency mixer thus represents the amplifier output power folded about the frequency at line centre of the radio frequency local oscillator. This is quite accurate for the $^{13}\text{CO}_2$ lines because their widths are considerably less than both the velocity shift and the width of the entire filter bank. The $^{12}\text{CO}_2$ line, however, is very broad and its interpretation is more difficult.

In a computer simulation of the Martian atmosphere we assumed an atmosphere of 100% carbon dioxide consisting of plane-parallel layers of constant temperature and pressure (Fig. 3). The rate of change of temperature with altitude is conventionally defined as the lapse rate, and the height at which the pressure drops by a factor of e is similarly defined as the scale height. We assumed a constant lapse rate and a scale height, at the surface, of 11 km. Parameters used in the simulation were:

T_s = surface temperature (given separately for each line in Fig. 3);

$\Delta T_{AS} = T_s$ minus the temperature of the atmosphere at the surface;

Γ = temperature lapse rate (degrees km^{-1});

P_s = surface pressure (mbar).

In addition we defined the parameter

$$z = [P_s / 6 \text{ mbar}] R(13/12) \cdot A(13/12).$$

This is proportional to the number of $^{13}\text{CO}_2$ molecules in the atmosphere and to the transition rate, where

$R(13/12)$ = ratio of isotopic species by number and

$A(13/12)$ = ratio of Einstein A coefficients for equivalent lines in the two isotopic species.

The best values of Γ , ΔT_{AS} , and z each depend to a large extent on which values are taken for the other two parameters (Table 1). This is because the number of absorbing molecules and their average temperature must remain nearly fixed in order to maintain the same absorption profile for each model. Four sets of these parameters which give equally good fits to the data are given in Table 1. Model 1 (Table 1) uses the adiabatic lapse rate for Mars. Higher lapse rates would be quickly reduced to the adiabatic case by convection. Model 4, with zero lapse rate, is physically unrealistic but is included to show that z does not continue to decrease as the lapse rate further decreases. The results themselves do not put upper bounds on ΔT_{AS} and z ; values for z which fit the data are, however, in all cases larger than expected and values of ΔT_{AS} were therefore chosen so as to minimise z .

Statistical errors in the data produce uncertainties in the model. The standard deviation of T_s is about 1.5 K for the P(16) line and about 3.0 K for P(22) and P(26); systematic errors in the calibration cannot, however, be completely ruled out. The value of T_s is obtained from the black body calibration. Based on this calibration our measurement of the lunar flux is about 15% higher than the published value and has a standard deviation of 3% but the discrepancy is, at least in part, because of imprecise knowledge of the Sun angle at the point observed. In any case the discrepancy is in the direction which, if corrected for, would reduce our measured flux from Mars and lower the surface temperature. The correlations between Γ , ΔT_{AS} , and z make uncertainties in these quantities difficult to estimate, but the errors are considerably smaller than the range allowed from model to model. For example, the standard deviation of z in Model 3 for the P(16) line is about 0.004 if Γ and ΔT_{AS} are kept fixed.

Previous data⁷ can be compared with the parameters we have fitted to our data. The effective continuum temperature near the $^{13}\text{CO}_2$ lines is about 250 K (Fig. 3). Mariner 9 (ref. 7), looking at the sub-solar point as we did, obtained

a temperature near 11 μm of about 260 K. Mariner 9 also measured a temperature lapse rate of 2.5 K km^{-1} and an atmospheric temperature at the surface about 25 K cooler than the surface itself. These values are close to those used in Model 2 (Table 1). The parameter z is of particular interest because our data indicate a higher value than expected in all models. Some limits can be placed on the ranges of the three factors comprising z . Mariner 9 reported a surface pressure of 6 mbar with a range from 4 to 8 mbar because of terrain changes. Our results place an upper limit of 12 mbar because at this pressure the effects of collisional line broadening would have become apparent. A value of 0.24 s^{-1} was taken for the Einstein A coefficient of $^{12}\text{CO}_2$ (refs 8,9). The correct value for $^{13}\text{CO}_2$ may, however, be somewhat different because this coefficient is strongly influenced by the Fermi resonance between the lower level of the absorbing transition and the 02^0 vibrational level. Even a small change in the mass of the carbon atom affects the strength of this interaction. Measurements of the relative output power of carbon dioxide lasers operating on the two isotopic species suggest that the ratio $A(13/12)$ is about 2/3 (C. Freed personal communication). Taking a value for surface pressure of

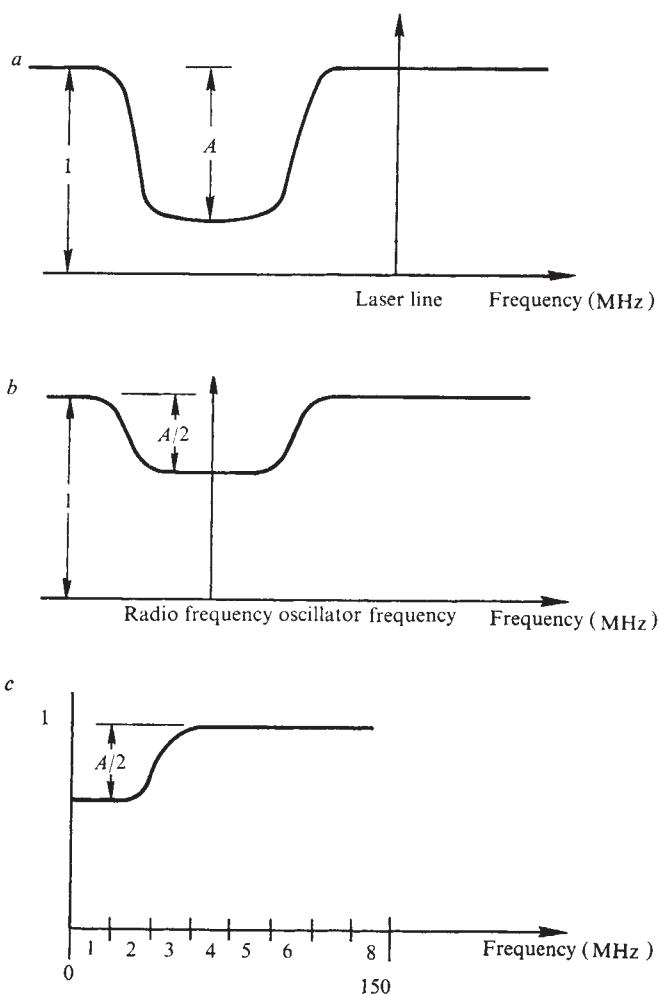


Fig. 2 Folding of the absorption line because of mixing: a, schematic representation of a Martian infrared absorption line shifted in frequency from a laser line of the same molecular transition by the relative motion of Earth and Mars. A is the fractional depth of the absorption. Infrared frequencies equally spaced on either side of the laser line appear at the same place in the radiofrequency spectrum of the amplifier output; b, the absorption line as it appears at the amplifier; c, the absorption line as it appears at the mixer output. The numbers along the abscissa represent the eight channels of the filter bank into which the mixer output is fed.

6 mbar, the terrestrial value of 0.011 for $R(13/12)$, and assuming a value of 2/3 for $A(13/12)$, gives a value of 0.007 for z , a number about three times smaller than those in Table 1 which fit our present measurements. The cause of this discrepancy is not clear.

Because $^{12}\text{CO}_2$ was expected to be 90 times more abundant than $^{13}\text{CO}_2$, we made the $^{12}\text{CO}_2$ measurements first. The results, however, were not as significant as those for $^{13}\text{CO}_2$. Because of the large width of the $^{12}\text{CO}_2$ line, measurements were made in bands 150 MHz wide distributed from 550 MHz below to 350 MHz above the expected frequency of the line centre. The bands could not be measured simultaneously because a suitably wide filter bank was not available. The measured flux was independent of frequency to within a standard deviation of 20%, indicating a line broader than the frequency range examined. The data are consistent with a continuum of 250 K folded into an absorption line with a flux of less than $2 \times 10^{-23} \text{ W Hz}^{-1}$ (200 K) and an equivalent width greater than 670 MHz (0.022 cm^{-1}). A computer simulation using a lapse rate of 2.5 K km^{-1} , a value for ΔT_{AS} of 30 K, and a surface pressure of 6 mbar predicts an equivalent width of 400 MHz for the $^{12}\text{CO}_2$ line. The difficulties of accurate cali-

Table 1 Parameters for computer simulation

	Γ	ΔT_{AS}	z
Model 1	5 K km^{-1}	25 K	0.027
2	2.5 K km^{-1}	30 K	0.02
3	1.3 K km^{-1}	35 K	0.017
4	0 K km^{-1}	45 K	0.02

bration mean that the discrepancy is probably not significant, although its direction is similar to that found for $^{13}\text{CO}_2$, corresponding to excess absorption.

The results demonstrate that infrared heterodyne spectroscopy can be used in astronomy for spectral resolution exceeding by several orders of magnitude that obtained with conventional techniques, although the copper-doped germanium photomixer used had a sensitivity about 25 times less than the theoretical limit of $2 \times 10^{-29} \text{ W Hz}^{-1}$ for heterodyne detectors at 11 μm . Continuously tunable infrared lasers would allow high resolution of lines anywhere in the infrared band and perhaps the detection of absorption lines of interstellar gas against bright sources.

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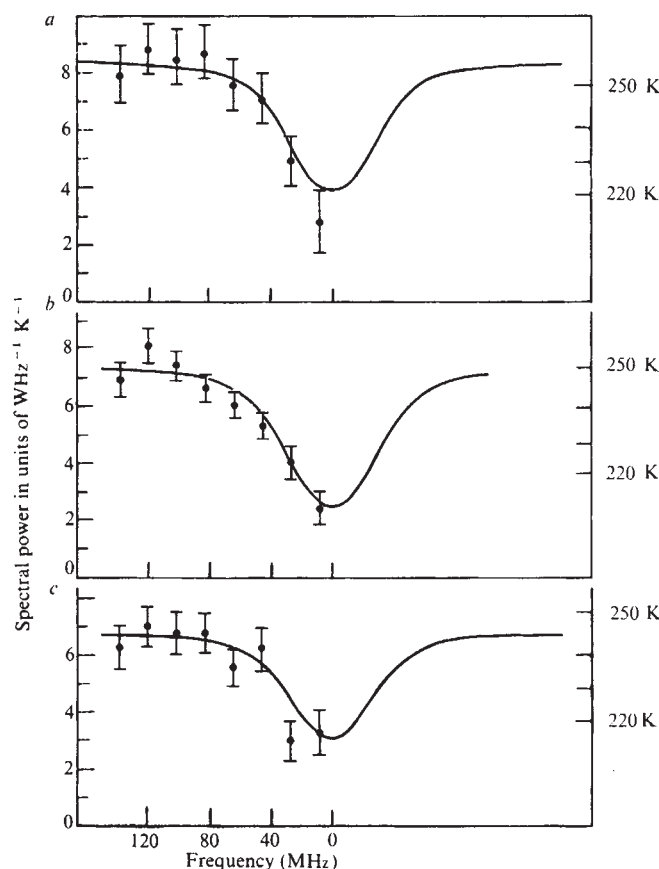


Fig. 3 Data obtained for the P(26) *a*, P(16) *b*, and P(22) *c*, rotational lines of $^{13}\text{CO}_2$. In *a*, $I_s=256 \text{ K}$, and equivalent width=50 MHz; *b*, $I_s=250 \text{ K}$, and equivalent width=66 MHz; *c*, $I_s=246 \text{ K}$, and equivalent width=49 MHz. The original data has been unfolded and plotted in the format of Fig. 2*a* as half of an infrared line. The ordinate is received spectral power in W Hz^{-1} divided by Boltzman's constant k and referenced to the top of the Earth's atmosphere. Absolute temperatures for black bodies of equal spectral radiance are shown at the right of the figure. Error bars represent plus and minus one standard deviation and correspond closely to predictions of the theory of heterodyne mixing¹⁰. The abscissa MHz is measured from the expected position of line centre. The P(26) and P(22) lines each represent about 1 h of observation, the P(16) line about 2 h. The solid curves represent absorption line profiles calculated in a computer simulation of the Martian atmosphere (see text).

Petrogenetic implications of argon isotopic evolution in the upper mantle

THE construction of models for the isotopic evolution of terrestrial argon (unlike those for strontium and lead^{1,2}), has been severely hampered by the fact that there are to our knowledge, no reported studies of the initial argon isotopic ratios in rocks of various ages. It has been estimated³ that the cosmic abundances of the stable argon isotopes are ^{40}Ar , 0.4%; ^{38}Ar , 14.1%; and ^{36}Ar , 85.6%. This gives a cosmic $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of 0.005, which may reasonably be assumed to approximate closely the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the solid Earth at the time of formation.

The reported abundances of the argon isotopes in the terrestrial atmosphere⁴ at present are ^{40}Ar , 99.600%; ^{38}Ar , 0.063%; and ^{36}Ar , 0.337%, giving an atmospheric $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of 295.5. This anomalous situation is clearly related to the production of radiogenic ^{40}Ar derived from the natural decay of ^{40}K in the Earth's crust and mantle. An increase of

Table 1 Measured variation in $(^{40}\text{Ar}/^{36}\text{Ar})_i$ ratios

Source	($\times 10^6$ yr) Age	$(^{40}\text{Ar}/^{36}\text{Ar})_i$
1	Historic	282.9 $\rightarrow$ 294.8
2	Historic	292.1 $\rightarrow$ 320.4
3	0.075 $\pm$ 0.061	295.2 $\pm$ 11.1
4	0.120 $\pm$ 0.011	289.8 $\pm$ 3.8
5	0.194 $\pm$ 0.056	293.2 $\pm$ 6.0
6	0.259 $\pm$ 0.035	289.5 $\pm$ 7.3
7	0.534 $\pm$ 0.145	282.6 $\pm$ 14.6
8	12.4 $\pm$ 0.3	302 $\pm$ 6
9	15.3 $\pm$ 0.2	295 $\pm$ 3
10	15.8 $\pm$ 1.3	293 $\pm$ 19
11	53.1 $\pm$ 2.5	289 $\pm$ 21
12	64.8 $\pm$ 3.1	263 $\pm$ 21
13	55.4 $\pm$ 1.2	287 $\pm$ 9
14	119 $\pm$ 4	275 $\pm$ 8
15	410 $\pm$ 4	183 $\pm$ 70
16	415 $\pm$ 4	224 $\pm$ 37
17	411 $\pm$ 3	196 $\pm$ 61
18	770 $\pm$ 14	131 $\pm$ 88

(1) Basalts⁸; (2) single basalt flow²⁶; (3) – (7) New Zealand volcanics²⁷; (8) East Iceland volcanics²⁸; (9) Continental volcanics²⁶; (10) North-west Iceland volcanics²⁸; (11) – (13) Faeroes basalts²⁹; (14) Plutonic minerals⁷; (15) Continental margin, volcanics³⁰; (16) Plutonic biotites³⁰; (17) Plutonic biotites^{30,31}; (18) Continental dolerite dykes³².

more than four orders of magnitude over the initial terrestrial $^{40}\text{Ar}/^{36}\text{Ar}$ ratio is indicated.

To demonstrate the isotopic evolution of argon in the solid Earth, we calculated the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios retained in analysed suites of mantle-derived igneous rocks, both volcanic and plutonic, of different ages (Table 1). The initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios were calculated in a manner exactly analogous to the determination of initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, the intercept of an argon isochron following the linear equation:

$$(^{40}\text{Ar}/^{36}\text{Ar})_{\text{total}} = [\lambda_e/(\lambda_e + \lambda_B)](^{40}\text{K}/^{36}\text{Ar})(\exp(\lambda t) - 1) + (^{40}\text{Ar}/^{36}\text{Ar})_{\text{initial}}$$

A plot of the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios obtained from eighteen suites of mantle-derived samples against their time of emplacement or extrusion (Fig. 1) strongly suggests that there has been an increase in the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratio with time. This we interpret as evidence for a significant increase in the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the mantle over the past 800 Myr.

Implicit in this interpretation is the assumption that a significant proportion of the ^{36}Ar observed in the mass spectrum of argon extracted from terrestrial rocks and minerals is derived from the source region of the analysed samples and does not represent an atmospheric contaminant. We are supported in this contention by reported analyses of lava flows⁵⁻⁸ which have shown that most of the so-called ^{36}Ar 'contamination' comes not from the extraction line but is contained within the rocks themselves and is released along with ^{40}Ar during fusion. The initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios preserved in igneous rocks seem therefore to be representative of the $^{40}\text{Ar}/^{36}\text{Ar}$ ratios in the source region of the parent magmas. The $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in any source region will increase with time at a rate dependent on the concentrations of potassium and ^{36}Ar , and the rate of argon release to the atmosphere.

Analyses of historically erupted lava flows^{5,6,8}, indicate initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios ranging from 282.9 to 350.0. Measured ratios greater than the present-day atmospheric ratio have been explained by the inclusion of xenoliths and xenocrysts in the analysed whole-rock samples but a satisfactory explanation has not been given for the lower ratios. The possibility of diffusion and fractionation of atmospheric argon into the cooling lava⁸ does not seem realistic to us. This variation of the observed $^{40}\text{Ar}/^{36}\text{Ar}$ ratios in modern volcanic rocks may be due to inhomogeneities in the upper mantle or to remelting of older gabbroic rocks in layer 3 as suggested for some Icelandic rocks⁹. We therefore conclude, from the reported analyses of modern basalts, that the average $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the upper mantle is at present not greater than the atmospheric ratio (295.5) and possibly a little lower.

A similarity between the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the upper mantle and the terrestrial atmosphere leads us to the conclusion that it is the Earth's mantle, not the crust, that is the major source of atmospheric argon. Despite the obvious generation of radiogenic ^{40}Ar in the potassium-rich crust of the Earth, there is little evidence that significant outgassing of crustal argon is occurring today. It has been variously suggested (see Rankama¹⁰, for a summary) that the ^{40}Ar present in the Earth's atmosphere has been gradually released from crustal rocks throughout geological time as a result of weathering processes¹¹ or volcanism¹². Alternative models involving major degassing of the crust and mantle during the first 10^9 years of the Earth's history have been suggested by Nicolet¹³ and Damon and Kulp¹⁴, while Turekian^{15,16} and Schwartzman¹⁷ advocated continuous transfer of argon from both the crust and mantle. Modern plate tectonic theory provides a viable mechanism for the continual release of argon from the mantle, by way of

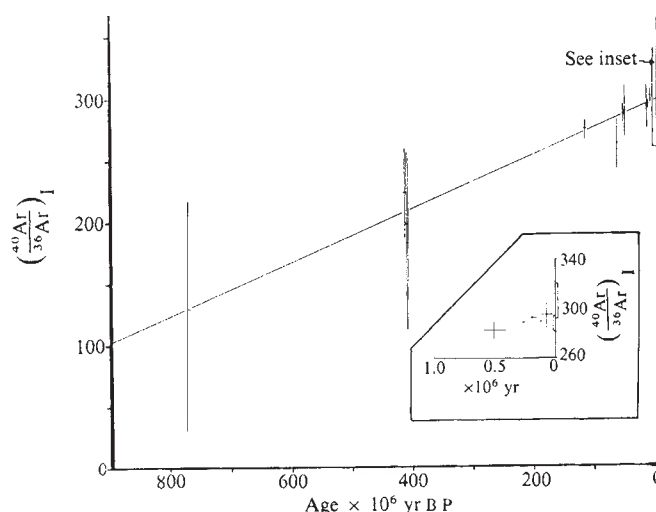


Fig. 1 Variation of the initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the upper mantle-derived igneous rocks over the past 800 Myr. This variation is considered to reflect the variation in argon isotopic ratio of the upper mantle region with time.

volcanism on active plate boundaries, but there is no simple mechanism available for the release of argon accumulating in the Earth's crust. Furthermore, a large component of crustal argon in the atmosphere would result in a present-day atmospheric $^{40}\text{Ar}/^{36}\text{Ar}$ ratio much greater than 295.5.

It is generally assumed that minerals melting and recrystallising in the Earth's crust under igneous and metamorphic conditions will lose argon to the atmosphere as a result of temperature-controlled diffusion processes. In reality, what is more likely to occur under these conditions is lattice diffusion and isotopic homogenisation of argon, with effective retention in the whole-rock system, a situation analogous to strontium isotopic homogenisation under similar conditions. Very little argon would escape from the crust directly to the atmosphere, except perhaps along major fracture zones. Homogenisation of the argon isotopes would have the effect of equalising the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the whole-rock system, so that initial $^{40}\text{Ar}/^{36}\text{Ar}$ ratios measured in crustal rocks should be greater than the present-day atmospheric ratio. Excessively high initial ratios have been recorded in metamorphic rocks, expressed in terms of an 'excess' ^{40}Ar concentration¹⁸⁻²¹.

To test our conclusions we have made a semiquantitative analysis of the available data. Any relevant calculations suffer, of course, from uncertainties in the estimation of potassium abundances^{22,23} in the Earth (Table 2). The calculated mass of radiogenic ^{40}Ar produced in the Earth from the decay of ^{40}K consequently has a large error which is propagated throughout the calculations.

The mass of ^{40}Ar produced in the Earth is calculated assum-

Table 2 Estimate of potassium and radiogenic argon in the Earth

	Mass of shell ²⁴ ($\times 10^{21}$ tons)	Potassium ^{22, 23} K % ($\times 10^{15}$ tons)	Radiogenic ⁴⁰ Ar ($\times 10^{15}$ tons*)
Crust	0.024	320 $\pm$ 55	1.350
Mantle and core	5.941	500 $\pm$ 200	0.008
Total	5.965	820 $\pm$ 207	0.014
Pyrolite	0.441	500 $\pm$ 200 (ref. 25)	0.108
Atmosphere			0.0653 (ref. 11)

* ⁴⁰Ar calculated from the potassium abundances with an age of 4,500 Myr for the Earth.

ing the Earth to be 4,500 Myr old. The observed mass of ⁴⁰Ar in the atmosphere (0.065×10^{15} tons), compared to the calculated mass of radiogenic ⁴⁰Ar produced in the Earth (0.11×10^{15} tons), suggests that only 60% of all the ⁴⁰Ar produced in the Earth has been released to the atmosphere.

In calculating the mass of ⁴⁰Ar in the mantle we make the following assumptions: (1) All ⁴⁰Ar in the atmosphere is derived from the solid Earth. (2) The upper mantle and crust are separate systems with respect to argon isotopic evolution. (3) Sediments make up approximately 5% of all crustal material²⁴, therefore not more than 5% of crustal argon has been released to the atmosphere and perhaps as little as 1% (ref. 10).

From assumption (3), taking 5% of crustal ⁴⁰Ar as being the maximum released to the atmosphere, the balance of the ⁴⁰Ar in the atmosphere is assumed to have come from the mantle, so (in units of 10^{15} tons):

$$^{40}\text{Ar in atmosphere} = (0.0653 \pm 0.0033)$$

$$^{40}\text{Ar released from crust (5\%)} = (0.0021 \pm 0.0004)$$

$$\therefore ^{40}\text{Ar released from mantle} = (0.0632 \pm 0.0034)$$

$$\therefore ^{40}\text{Ar remaining in mantle} = (0.0036 \pm 0.0269)$$

The large error in the estimation of ⁴⁰Ar remaining in the mantle arises because the abundance is the difference between two nearly equal and poorly known numbers.

For purposes of calculation, we now assume that: (4) All the potassium, and thus radiogenic ⁴⁰Ar, is concentrated in an upper mantle region with a pyrolite²⁵ composition. (5) This pyrolite layer is approximately 200 km thick and has a mass of 0.441×10^{21} tons (Table 1). (6) The argon isotopic composition is homogeneous throughout the pyrolite layer.

The maximum concentration of ⁴⁰Ar at present in this pyrolite layer can now be calculated knowing the mass of the layer and the mass of ⁴⁰Ar remaining in the upper mantle. Thus the maximum ⁴⁰Ar concentration in the total pyrolite layer is $(8.2 \pm 61.0) \times 10^{-3}$ p.p.m. ⁴⁰Ar.

The present-day ³⁶Ar concentration in the upper mantle can be estimated from the observed ³⁶Ar concentrations in modern volcanic rocks derived from the mantle. The concentration of ³⁶Ar in young volcanic rocks, measured in our laboratory, after correcting for line blank, is estimated to be $(2.4 \pm 1.1) \times 10^{-5}$ p.p.m. ³⁶Ar. Because these rocks have been at least partially outgassed on extrusion, this value serves at least as a minimum concentration for the ³⁶Ar in the upper mantle source region.

Assuming that the present-day ⁴⁰Ar/³⁶Ar atomic ratio in the upper mantle source region is 295 ± 10 (or 327 weight ratio), then the present-day ⁴⁰Ar concentration in the upper mantle is at least $(7.8 \pm 3.6) \times 10^{-3}$ p.p.m. ⁴⁰Ar. This value is close to that estimated in the pyrolite layer by the difference method. This near coincidence could be due to the cancelling out of the different assumptions (1) to (6), or it might indicate that Hurley's potassium abundances^{22, 23} are more precise than indicated in Table 2 and that most of the ⁴⁰Ar at present in the atmosphere has been released from the mantle.

According to our model, approximately 95% of all the ⁴⁰Ar produced in the mantle has been lost to the atmosphere. At least that percentage of ³⁶Ar must also have been lost, assuming no isotopic fractionation. From assumptions (5) and (6), using the observed concentration of ³⁶Ar in mantle-derived volcanic rocks, the mass of ³⁶Ar in the pyrolite shell (200 km thick) is estimated to be $(1.05 \pm 0.50) \times 10^{10}$ tons at present.

This must represent at most 5% of the ³⁶Ar originally present in the mantle. Therefore, 4,500 Myr ago, the mass of ³⁶Ar in the mantle was at least $(21.0 \pm 9.0) \times 10^{10}$ tons. There is at present 20.0×10^{10} tons of ³⁶Ar in the atmosphere, so it is quite reasonable to postulate that the majority of ³⁶Ar in the atmosphere has also come from the mantle.

Protocrustal material, with an average potassium concentration of 1.350%^{22, 23}, would yield in 4,500 Myr a concentration of ⁴⁰Ar ten times greater than that in the upper mantle. The net result of this would be to give ⁴⁰Ar/³⁶Ar ratios in the deep crust that are an order of magnitude greater than the ⁴⁰Ar/³⁶Ar ratio in the upper mantle.

Initial ⁴⁰Ar/³⁶Ar ratios on igneous rocks which have not undergone isotopic homogenisation since crystallisation should yield information about the source region. It is thus possible to distinguish clearly between partial melting in a low potassium environment (such as the upper mantle) and partial melting of old sialic material. Initial ⁴⁰Ar/³⁶Ar ratios should also be very sensitive to any geological contamination (assimilation) of a basaltic magma by older potassium-rich material.

In summary: (1) Initial ⁴⁰Ar/³⁶Ar ratios of mantle-derived igneous rocks have increased steadily over the past 800 Myr. (2) The present-day ⁴⁰Ar/³⁶Ar ratios in the upper mantle and atmosphere are very similar. (3) The majority of argon at present in the atmosphere has been derived from the mantle rather than the crust. (4) Initial ⁴⁰Ar/³⁶Ar ratios on igneous rocks are potentially more sensitive petrogenetic indicators than strontium or lead isotopes.

Calculation of argon isochron parameters from the literature suffers from several omissions in the presentation of K-Ar age data for publication and we suggest the following minimum list of items that need to be reported: (1) The measured argon ratio in air. (2) The system line blank and isotopic ratio. (3) The weight of sample fused. (4) The concentration of ³⁶Ar measured or the percentage of 'atmospheric' to 3 significant figures.

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Plate tectonics, volcanism and the lithosphere in British Columbia

THE most important feature of the present tectonic situation off the west coast of Canada is the ridge-trench-fault type triple junction between the Juan de Fuca, Pacific and American lithospheric plates near the continental margin, at approximately 51°N (Fig. 1). The half spreading rate for the Juan de Fuca Ridge, based on the magnetic lineation pattern, is 2.9 cm yr⁻¹, and the calculated rate of motion along the Queen Charlotte transform fault between the American and Pacific Plates is 6 cm yr⁻¹ (ref. 1). The main part of the Juan de Fuca Ridge is probably spreading parallel to the north-east trending Blanco fracture zone which marks the southern edge of the plate at approximately 43.5°N. The vector diagram for the relative motion of the three plates during the late Cainozoic suggests

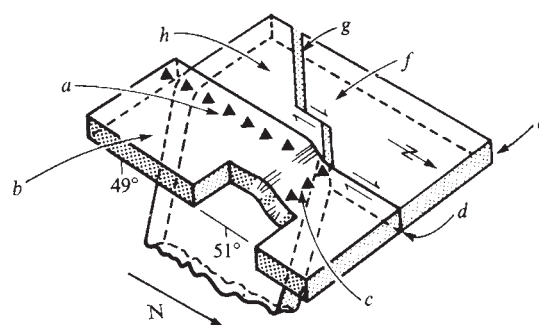


Fig. 2 The Canadian Cordillera and the adjacent north-east Pacific Ocean viewed from the north-east. a, 'arc' volcanics over the subducted part of the Juan de Fuca Plate; b, American Plate; c, lithosphere; d, Queen Charlotte Transform Fault; e, Pacific Plate; f, east-west volcanic lineaments; g, Juan de Fuca Ridge; h, Juan de Fuca Plate. Note the flexing of the American plate and the associated east-west topographic and volcanic lineaments (f) at approximately 51°N; the oblique underthrusting of the Pacific Plate along the Queen Charlotte Fault (d), and the associated north-south volcanic belt of British Columbia north of 53°N.

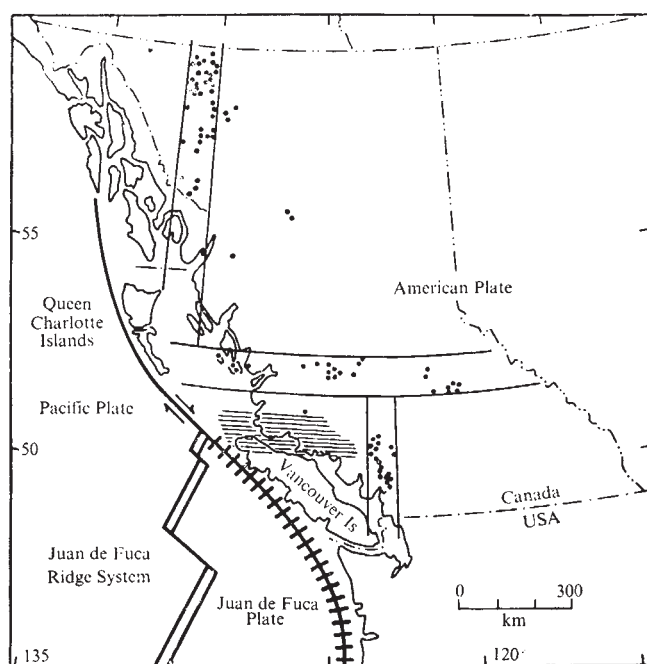


Fig. 1 Plate boundaries (after Atwater¹) and the volcanic (after Souther²) and topographic lineaments of the Canadian Cordillera. ●, Volcanic centres; double heavy lines, ridges; heavy, crossed, lines, trench; heavy line with reversed arrows, transform fault; thin close-set lines, east-west topographic lineaments.

that the Juan de Fuca Plate is being thrust obliquely below the American Plate at 2.5 cm yr⁻¹ in a direction of N 35° E (ref. 1).

Travel-time anomalies for the Seattle earthquake which occurred at a depth of 60 km, indicate the existence of a high velocity slab dipping eastwards at 50° beneath south-western Canada and the north-western United States². The magnetic anomalies of the Juan de Fuca Plate continue across the continental slope, and there is evidence of steep reverse faulting on the slope parallel to the continental margin³. Both these lines of evidence indicate recent subduction of the Juan de Fuca Plate below the American plate, as does the presence, further inland, of the late Cainozoic andesitic volcanic belt of the Cascades.

In the absence of a Benioff seismic zone, however, it is difficult to say how far the subducted Juan de Fuca Plate may extend to the east below the Cordillera. I here suggest that a number of phenomena within the Canadian part of the Cordillera, in the vicinity of the triple junction between the Juan de Fuca, Pacific and American Plates, can be explained if the influence of the subducted plate extends at least 500 km eastwards from the continental margin.

The most obvious physiographic feature of the Canadian Cordillera is that the area south of 51°N, the latitude of the triple junction, is approximately 500 m higher than that to the north. Geologically, there is more granitic 'basement' exposed to the south than there is to the north⁴, and in the eastern metamorphic belt of the Cordillera, the highest grades of metamorphism are again south of 51°N and decrease rapidly northwards⁵. These features all suggest that there has been a greater degree of uplift and erosion south of 51°N.

More than 90% of the approximately 150 Pleistocene and Recent volcanic centres which have been identified in the Canadian Cordillera⁶, fall within the two north-south belts and one east-west belt (Fig. 1). The volcanic centres are generally short-lived cinder cones with alkali olivine basalt flows, but at least 20 are composite volcanoes with flows ranging from picrite basalt, through alkaline andesite and dacite, to rhyolite. The north-south line of volcanic centres in southern British Columbia could be the northern continuation of the Cascade andesitic volcanic centres, and part of a volcanic arc over the subducted portion of the Juan de Fuca Plate. The greater arc-trench distance in Canada may indicate that the plate dips eastwards at a shallower angle north of 49°N. The east-west volcanic belt is at approximately the same latitude as the triple

junction on the continental margin, and the belt may be related to an upward bending of the American Plate over the northern edge of the Juan de Fuca Plate⁷, with magma generated by partial melting in the zone of tension at the base of the lithosphere (Fig. 2). South of the east-west volcanic belt, the reverse bend causes tension in the upper part of the lithosphere. The consequent east-west fracturing has been emphasised by subsequent erosion, producing the prominent east-west lineations in the Coast Mountains (Fig. 2). Preliminary interpretation of gravity data over Queen Charlotte Sound suggests that the east-west topography of the Coast Mountains continues below the sedimentary cover of the continental shelf north of Vancouver Island. The oldest identified sediments overlying this topography are late Miocene⁸, which indicates that the triple junction to the west has remained at approximately the same latitude for the last 15 Myr.

The model in Fig. 2 also relates the north-south volcanic belt of north-western British Columbia to a lithospheric flexure, in this case caused by the oblique convergence of the Pacific and American Plates. Data from this northern region are scarce, but one fault-plane solution for the 1949 Queen Charlotte earthquake (54.1°N, 132.6°W) requires right lateral slip, with some underthrusting of the Pacific Plate below the American Plate⁹. Seismic profiling across the southern end of the Queen Charlotte Fault (52°N) has shown a raised block of oceanic crust at the foot of the continental slope¹⁰. It is possible that this block was raised by reverse faulting similar to that found along the continental slope further south, where the faulting is believed to be related to subduction of the Juan de Fuca Plate below the American Plate⁸. In the St Elias mountains near the junction of the borders of Alaska, Yukon and British Columbia, there are marine Pliocene sediments which have been raised at least 6,400 m since deposition⁶. Thus, there is evidence that the Pacific Plate has been thrust obliquely under the American Plate, with the western margin of the latter raised to the north of the triple junction (Fig. 2). Tension at the base of the bent plate could have caused the observed volcanic lineament. With this interpretation the Queen Charlotte Fault cannot be regarded as a true transform fault (compare with Fig. 1) because of the element of convergence between the Pacific and American Plates.

The half wavelength of the flexure in the American Plate between the east-west volcanic belt and the parallel zone of topographic lineaments to the south, is 200 km and, assuming that the density between the lithosphere and the supporting asthenosphere is 3.3 g cm^{-3} and $g = 980 \text{ cm s}^{-2}$ the apparent flexural rigidity of the plate¹¹ will be $1.3 \times 10^{22} \text{ Nm}$. The thickness of the lithosphere¹¹, using a Poisson's Ratio of 0.25 and a Young's Modulus of $7 \times 10^{10} \text{ N m}^{-2}$ will then be 28 km. A similar value for the thickness of the lithosphere has been obtained from a study of the deformation of glacial lake shorelines by Fulton and Walcott¹² who estimated the lithosphere in the Cordillera south of 51°N to be 20–50 km thick. Even within these wide limits, the lithosphere is considerably thinner than the 110 km found below stable areas of the crust.

The concept of an abnormally thin lithosphere below the southern part of the Canadian Cordillera supports earlier ideas. For instance, Caner¹³ invoked a layer of possible partial melting in the lower crust to account for anomalous variations in the Earth's magnetic field in the south-eastern part of the Cordillera. Wickens¹⁴ has shown that the low velocity layer equivalent to the asthenosphere, approaches the base of the crust below the southern part of the Cordillera. The thickness of the lithosphere is approximately equal to the depth of the M discontinuity derived from refraction seismic data, suggesting that the upper surface of the asthenosphere approaches the base of the crust in this part of the Cordillera. The P_n velocity over the subducted part of the Juan de Fuca Plate is low (approximately 7.8 km s^{-1} , Berry, personal communication, 1973) and I have concluded⁷ that the density of the upper mantle below the central part of the Cordillera has also to be lower than normal in order to reconcile the gravity and refrac-

tion seismic results. As may be expected from the model in Fig. 2, P_n for the central part of the Cordillera increases to a more normal value of 8.1 km s^{-1} north of the latitude of the triple junction and the area overlying the Juan de Fuca plate.

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Isotopic analysis of the deep structure in the Tyrrhenian Sea

THE monotonic distribution of temperature and salinity with depth, observed in most oceans of the world is replaced, in certain areas, by a peculiar 'staircase' structure in which sheets of water of constant temperature and salinity are separated by comparatively thin layers, across which there is a sharp gradient. Although its origin is speculative, the structure is often associated with the intrusion of warm saline water into a water mass that is colder and fresher. The structure is present in the eastern Atlantic, west of Gibraltar, below the characteristic Mediterranean outflow^{1,2}, and in the Tyrrhenian Sea below the inflow of the Levantine Intermediate Water through the Strait of Sicily^{3,4}.

We have attempted to distinguish the layers in the Tyrrhenian Sea by isotopic analysis of water samples collected from the different layers.

The Mediterranean is divided into two main basins by the Strait of Sicily. The eastern part is referred to as a 'concentration basin': the amount of water lost by evaporation exceeds the amount gained by precipitation and river discharge. In order to maintain the water and salt balance, two opposite flows connect the eastern and western basins through the Strait of Sicily. The upper flow, towards the east, carries in more 'Atlantic' water of lower salinity than the opposite flow at the bottom^{5,6}.

The deeper flow, the Levantine Intermediate water, is characterised by a salinity of about 38.7‰ and a temperature of about 14° C, and, after passing the sill, descends to a level of stability at a depth of about 600 m in the Tyrrhenian Sea. As it extends northwards and eastwards, it can be identified by the observed maxima in temperature and salinity profiles. It is the water mass below this maximum which, at some locations, exhibits the 'staircase' structure, although at other locations the more normal monotonic decrease to the bottom is observed.

In May 1972, samples were taken from 24 stations in the Tyrrhenian Sea, using a salinity-temperature-density (STD) probe. In addition, Nansen casts immediately followed the STD casts in five of the locations. At the station which exhibited the most pronounced structure, samples were taken at six depths below the salinity maximum (Fig. 1).

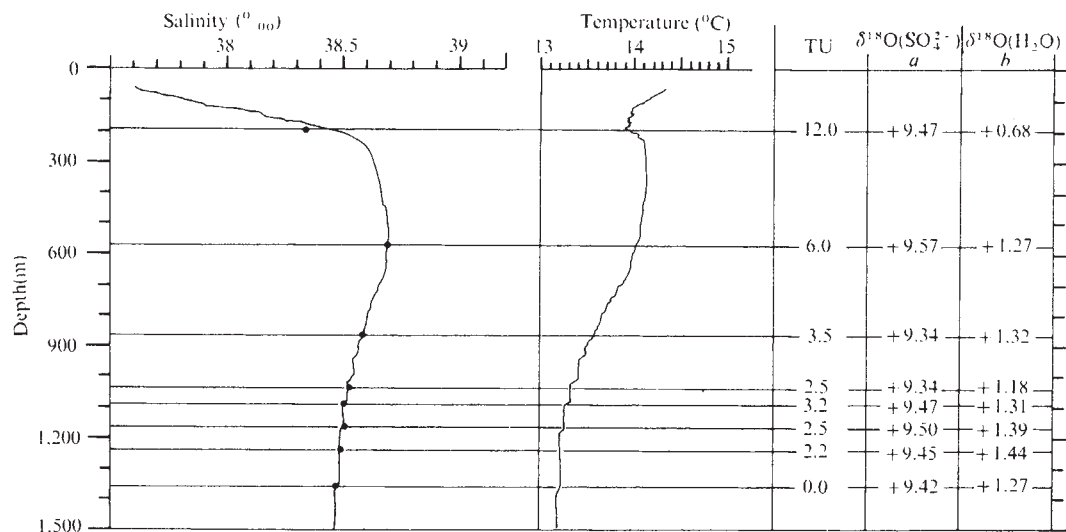


Fig. 1 Salinity, temperature and isotopic profiles at hydrographic station 19B1 in the low Tyrrhenian sea (39° 59.0'N, 12° 42.5'E). a, b, results relative to SMOW.

The isotopic analysis concerned the concentration of the stable ^{18}O content of water and of the dissolved sulphate, and the tritium concentration in superficial and deep seawater samples.

The procedure used in the preparation of samples for measuring the $^{18}\text{O}/^{16}\text{O}$ ratios in sulphate ions has been described⁷. The oxygen isotope analyses were carried out using the graphite-reduction technique⁸.

Oxygen isotope ratios are given in δ notation:

$$\delta^{18}\text{O} = \{[(^{18}\text{O}/^{16}\text{O}) \text{ sample}/(^{18}\text{O}/^{16}\text{O}) \text{ standard}] - 1\} \times 10^3$$

The results are reported relative to Standard Mean Ocean Water (SMOW)⁹. Isotopic analyses of the CO_2 were made using an ATLAS M86 mass spectrometer. The expected error of the $\delta^{18}\text{O}(\text{SO}_4^{2-})$ measurements was estimated at $\pm 0.07\text{‰}$.

Water oxygen isotopic samples were prepared using the isotopic equilibration technique¹⁰. The expected error of the $\delta^{18}\text{O}(\text{H}_2\text{O})$ measurements is $\pm 0.1\text{‰}$.

Tritium is formed in the atmosphere by, essentially, three mechanisms: the interaction of the nucleonic components of the cosmic radiation with the constituents of the atmosphere; direct origin from the Sun; and thermonuclear weapon tests. Most of the atmospheric tritium will form water and enters the oceans as tritiated water by molecular exchange across the air-water interface.

The concentrations are reported as TU (tritium unit); that is, the number of tritium atoms relative to 10^{18} hydrogen atoms¹¹. The accuracy of the tritium concentration measurement is approximately ± 1 TU.

Table 1 presents the results of the isotope analysis together with the observed temperature and salinity of the samples. All

Table 1 Hydrological stations, STD and isotopic results

	Depth (m)	Temperature (°C)	Salinity (‰)	TU	$\delta^{18}\text{O}(\text{SO}_4^{2-})^*$	$\delta^{18}\text{O}(\text{H}_2\text{O})^*$
	0		37.685(B)†			
Station 15 (38° 27.8'N, 11° 16.2'E)	46	15.61		8.0	+9.65	
	267	13.93		7.7	+9.49	
	746	13.29		1.5	+9.31	
	975	13.09	38.466			
	0		37.512(B)		+9.64	
Station 17 (39° 24.4'N, 11° 43.0'E)	150	13.67	38.298	9.5	+9.38	
	450	14.06	38.705	6.0	+9.42	
	998	13.28	38.515	0.0	+9.45	
	0		37.494(B)	12.5	+9.38	+0.69
	191	13.86	38.334	12.0	+9.47	+0.68
	571	14.03	38.689	6.0	+9.57	+1.27
Station 19B1 (39° 59.0'N, 12° 42.5'E)	864	13.60	38.589	3.5	+9.34	+1.32
	1,039	13.36	38.530(STD)	2.5	+9.34	+1.18
	1,092	13.30	38.511	3.2	+9.47	+1.31
	1,165	13.31	38.510	2.5	+9.50	+1.39
	1,236	13.25	38.492	2.2	+9.45	+1.44
	1,355	13.21	38.476	0.0	+9.42	+1.27
	0		37.412(B)			
Station 21 (40° 27.8'N, 12° 37.6'E)	49	14.57	37.552	8.0	+9.41	
	521	14.03	38.699	5.0	+9.50	
	1,175	13.25	38.497	1.0	+9.38	
	1,430	13.19	39.477	0.0	+9.39	
	0		37.554(B)			
Station 24 (41° 17.7'N, 11° 03.6'E)	179	13.86	38.440	9.5	+9.45	
	525	13.92	38.665	4.0	+9.41	
	846	13.53	38.572	2.0	+9.50	
	992	13.29	38.510	0.0	+9.47	

* Results relative to SMOW.

† (B), Bucket samples from the surface.

STD casts were made with a Nansen bottle attached above the probe for calibration purposes. Corrected depths were calculated using unprotected thermometers.

The $\delta^{18}\text{O}(\text{SO}_4^{2-})$ values range from 9.31‰ to 9.65‰, with an average value of 9.44‰. This agrees well with a previously reported value of 9.47 ± 0.19 ‰ from the Tyrrhenian Sea¹². In the region of pronounced step structure (Station 19B1) the $\delta^{18}\text{O}(\text{SO}_4^{2-})$ values lie between 9.57‰ at 547 m depth and 9.34‰ at 900–1,000 m.

The $\delta^{18}\text{O}(\text{H}_2\text{O})$ analysis, made only at station 19B1, showed an increase from a value of about 0.7‰ in the surface waters to a value of about 1.3‰ in water below 600 m.

A net decrease of tritium concentration with depth can be seen at all stations. The tritium concentration in the thermocline area (Station 19B1) is very uniform (about 12 TU) and considerably higher than in subjacent waters. In the intermediate Levantine water (about 600 m) the tritium concentration is 6 TU.

In the region of the stepped structure the tritium content decreases with depth (2.8 ± 0.6 TU), but the disparity is a little lower than the expected error. The deepest water sample is 'dead'.

It must be admitted that the results of this first attempt to detect significant differences in the isotope concentration of the separate layers are inconclusive. There is no doubt that both in the layered structure and at adjacent stations where the layering is not marked, the tritium concentration decreases with depth, and the tritium contents in the stepped portion of the TDS profile at station 19B1 may be taken as an indication that the observed steps concern a water mass showing a uniform apparent age, intermediate to those of the overlying Levantine and underlying deeper waters. If, as is probable, the stepped water mass results from mixing between the Levantine Intermediate water and the deep, 'dead', water, then at different times correspondingly different degrees of mixing and, consequently, different tritium concentrations will occur. Thus, because they have the same tritium contents, the observed layers may be formed at the same time or at very close times. There is also some indication that the tritium concentration of the Levantine Intermediate water (300–600 m) decreases to the north, though again, the differences are roughly comparable to the accuracy of the measurements.

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Ophiolites and oceanic crust

OPHIOLITES consist of a pseudostratiform sequence, of harzburgite, tectonite, ultramafic and mafic cumulates sometimes including gabbro and quartz diorite (plagiogranite) intrusions, dolerite dyke swarms, pillow lava¹, and deep-sea sediments^{2–4}. This assemblage occurs in all Phanerozoic mountain systems and is interpreted as fossil oceanic crust and uppermost mantle^{5–10}. Outstanding problems include differences between the chemical properties of ophiolites and rocks thought to represent present-day oceanic crust^{11,12}, the lack in some complexes of recognised dyke swarms or cumulates, and the relative thinness of ophiolite mafic rocks compared with standard oceanic crustal sections^{5,8,13}.

We here attempt to resolve some of these difficulties by comparing data on oceanic crustal structure, with data on selected, relatively complete, ophiolite complexes. We also discuss possible relationships between age, spreading rate, and crustal structure, and suggest a more precise correlation between oceanic crustal and ophiolitic layers.

Table 1 presents data on both the internal structure of ophiolite complexes used in this comparison, and our inferred thicknesses of oceanic layers 2 and 3 (see refs 13 and 14).

Several workers have discussed the variation of oceanic crustal structure with age and rate of spreading^{15–17}. Sutton *et al.*¹⁸, reported a series of combined wide-angle reflection-refraction measurements from the Pacific Ocean, which present a somewhat different structural picture from the normal oceanic model. In particular they found abundant evidence for a basal layer of $V_p = 7.1–7.6$ km s⁻¹ between the main oceanic layer (layer 3) and the mantle. They suggested that this layer is widespread, but seldom recognised. We here compare their results with the ocean crustal ages¹⁹ and spreading rates²⁰.

The thicknesses reported by Sutton *et al.*¹⁸ show little relationship to age of crust (Fig. 1). This contrasts with observed increases in the thickness of layer 3 (ref. 17) but it may be because of inadequate data.

Figure 2 shows that the thicknesses of the oceanic and the basal layer, respectively decrease and increase as the spreading rate increases. Comparison of seismic velocity (V_p) with spreading rate (Fig. 3) reveals little change in the oceanic layer but an apparent decrease with increasing rate in the basal layer.

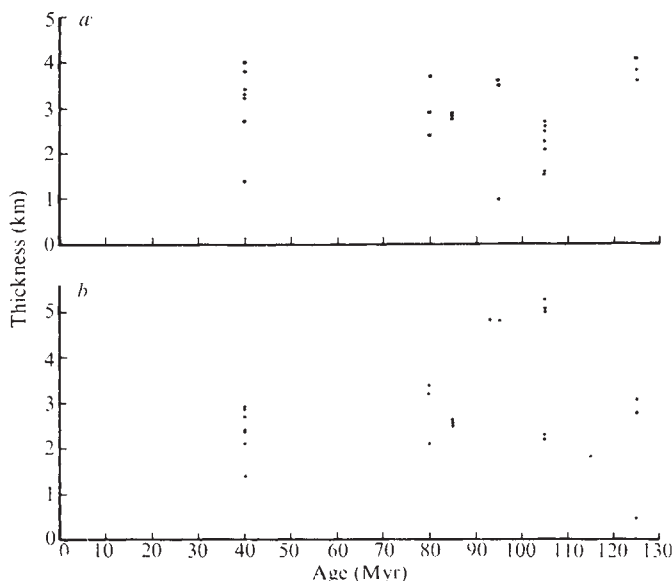


Fig. 1 Relationship between the thicknesses of the oceanic (a, $V_p = 6.2–7.0$ km s⁻¹) and basal (b, $V_p = 7.1–7.8$ km s⁻¹) layers¹⁸ and the age of the crust.

Table 1 Age, thickness, and structure of selected ophiolite complexes

Complex	Thickness (km)				Oceanic correlation		
	Transition	Gabbro	Diabase	Lava	Layer 3	Layer 2	Total
Vourinos ⁴ (V)	1	1.6	~0.5	~0.5	1.6	1	2.6
Troodos ^{13,26} (T)	0.5	0.5	2.3	0.9	0.5	3.2	3.7
Kizil Dag ²⁷ (KD)	2.0	2		0.4	0-2	0.4-2.4	2.4
Semail ^{6,7} (S)		3	2	1	3	3	6
Othris ²⁸ (O)		0.25		0.6	0-0.25	0.6-0.85	0.85
Pindos ^{25,27} (Pi)		~1	~1	0.3	1	1.3	2.3
Papua ³⁰ (Pa)	0.5	4		4-6	4	5±1	9
Elba ^{31,32} (E)		0.1	1.1		0.1	1.1	1.2
Betts Cove ³³ (BC)		0.2	1	1	0.2	2	2.2
Canyon Mountain ⁵ (CM)		3.1		0.4	3.1	0.4	3.5
Bay of Islands ⁸ (BI)	0.8	2.6	0.6	1.2	2.6	1.8	4.4

The thicknesses of the units are only approximate. They are either taken from the references indicated, or measured from published cross sections.

The sections of Sutton *et al.*¹⁸ closely resemble those of the selected ophiolite complexes (Fig. 4). The range of thickness of the oceanic layer (approximately 1-5 km), is surprisingly similar to the inferred range of thickness of layer 3 (gabbro) in ophiolites (0.25-4 km).

Furthermore, the presence of a basal layer intermediate in velocity between the oceanic layer and the mantle, also has a geological counterpart in ophiolite complexes, that is, the mixed mafic-ultramafic or 'transition' zones of many

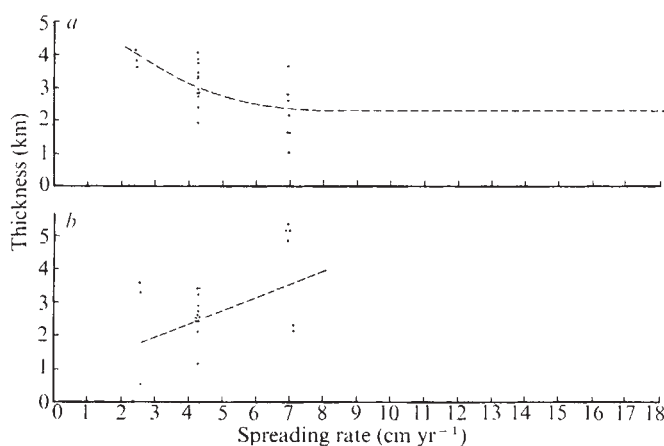


Fig. 2 Relationship between the thicknesses of the oceanic (a) and basal (b) layers¹⁸ and the spreading rate.

ophiolites. These zones are composed principally of layered cumulates, which in at least one case (Vourinos) are arranged in cyclic units²¹. The interstices between cumulus crystals (for example, olivine) may be filled by material of the same composition as the crystal itself²² (thereby forming, for example, a dunite); or they may be filled by other minerals (for example, plagioclase and pyroxene)²² thereby forming, for example, a plagioclase hertzsolite or wehrlite. As the postcumulus material comprises as much as 50% of a cumulate, fairly large differences of seismic velocity can be expected, depending on the relative amounts of 'secondary enlargement space filling' material.

These differences are of fundamental importance in any consideration of magmatic processes at a ridge, because 'secondary enlargement' apparently is controlled by the rate of accumulation of cumulus crystals in layered cumulus complexes²³. When the rate of accumulation is slow, diffusion operates to produce enlargement. When the rate is rapid, the original magma effectively is trapped and 'space filling material' is formed. This relationship is evident in the Stillwater complex where there is an inverse relationship between the thickness of section of olivine cumulate and the amount of enlargement²⁴.

Thus, the data suggest that in slow spreading ridges,

there is slow cooling, with a lot of diffusion and secondary enlargement, resulting in a thinner, higher velocity, basal layer, and a thicker, lower velocity, oceanic layer. Data from fast spreading ridges suggest that cooling is faster, with less diffusion, and a high degree of space filling, giving rise to a thicker, lower velocity, basal layer, and a thinner, higher velocity, oceanic layer (see Figs 3 and 4). It must be emphasised, however, that the seismic data are sparse and highly variable and that the mixed mafic-ultramafic zones of many ophiolites are complicated by pyroxenite and gabbro dykes, and above all, are commonly partially serpentinised. It is possibly these geological features which give rise to the scatter in Figs 1-3.

Some olivine cumulates in the Pindos complex²⁵ contain as much as 25% plagioclase and 25% pyroxene, whereas olivine cumulates in the Vourinos complex²¹ are essentially monomineralic (dunites). These relationships imply that primary cumulus processes are first order determinants of oceanic and basal layer thicknesses and velocities. Pindos may have formed from a faster spreading ridge than Vourinos.

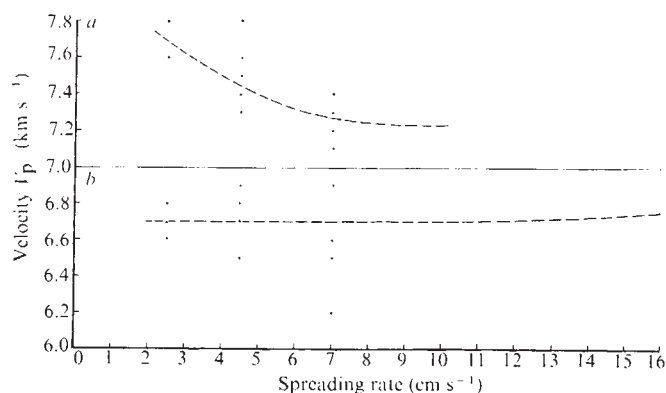


Fig. 3 Relationship between velocity (V_p) in basal (a) and oceanic (b) layers and spreading rate.

Completely unserpentinised ultramafics are rare in ophiolite complexes, but occur in a few, such as Vourinos or Papua. Possibly the serpentinised-fresh ultramafic contact in these complexes represents the seismic Moho rather than the mafic-ultramafic contact. Whether serpentinised or not, it is apparent that the basal layer-mantle interface may not correspond to the magmatic-metamorphic contact of ophiolites^{1,21}.

It is not yet possible to infer a spreading rate for a given ophiolite complex by comparing the internal structure and petrology with seismic data of oceanic crust, but some trends are emerging. It seems reasonable to assume, however, an oceanic crustal model as follows: layer 2=pillow lava, and dolerite, or zeolite-greenschist

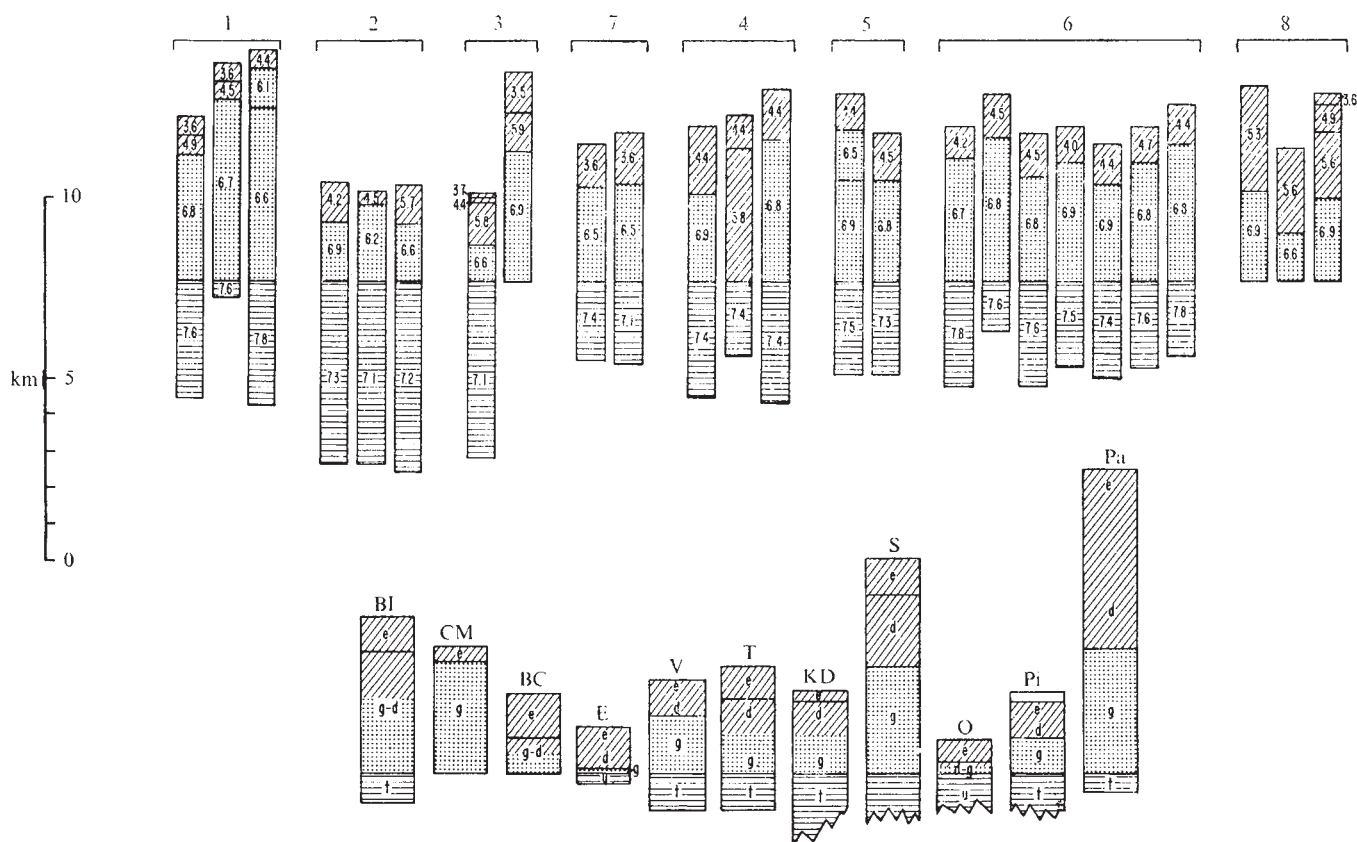


Fig. 4 Upper row, crustal sections of the Pacific basin¹⁸. Numbers over sections are sites of Sutton *et al.*¹⁸. Numbers within sections are seismic velocities (km s^{-1}). Lower row, selected ophiolites complexes, e, Extrusives, generally mafic pillow lava; d, dolerite; g, gabbro; +, mixed mafic-ultramafic or 'transition' zone. Symbols for ophiolite complexes are from Table 1.

facies equivalents^{15,16}; oceanic layer (layer 3)=gabbro, and quartz diorite (plagiogranite) and/or amphibolite; basal layer=a mixed ultramafic cumulate zone together with partly serpentinised tectonite.

Refinement of this model awaits more data on ophiolite, oceanic crustal lithologies, and thicknesses and elastic properties, obtained either seismically or by drill sampling. The elastic properties of ocean ridge dredge samples confirm that layer 2 is basaltic and the oceanic layer (layer 3) is gabbroic, but identification of basal layer rocks is unlikely using this approach, and coherent stratigraphy is difficult to establish in dredge hauls. The ultimate aim is the calibration of ophiolite spreading rates, and thus those of now vanished Phanerozoic oceans.

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Optical absorption phenomena at electrode surfaces

THERE has been a considerable expansion recently, in the application of optical techniques, such as internal reflection and specular reflection spectroscopy, ellipsometry, interferometry, holography and optical microscopy, to the study of adsorption phenomena in electrochemical reactions¹⁻³.

In general, these methods have been used to study the optical properties of the electrode material and of thin films adsorbed on to their surfaces. Internal reflection spectroscopy and specular reflection spectroscopy have both been used to monitor the surface concentration of an electroactive species⁴⁻⁶.

We describe here some preliminary observations of the optical absorption phenomena which occur above a platinum cathode surface when dilute aqueous solutions of metal cations containing a preponderant concentration of an indifferent electrolyte, such as an alkali metal halide, are electrolysed at low current densities and controlled potentials in a silica cuvette.

Well defined transient absorption spectra are obtained both 'on' the cathode surface and as much as 10^6 – 10^7 Å into the solution phase. The wavelengths of the absorption maxima of the transient absorption bands bear a close relationship to the shorter wavelength absorption lines of the same atomic species in a gas phase. Furthermore, the peak intensities of these transient signals are proportional, in each case, to the concentration of the free electroactive cations in solution. No corresponding signals are observed at the anode or in the absence of the electroactive species, or at potentials below that required to discharge the particular cation. The signals are most intense at grazing incidence on the cathode, but they are also found in the solution phase when the cathode is below the analysing light beam.

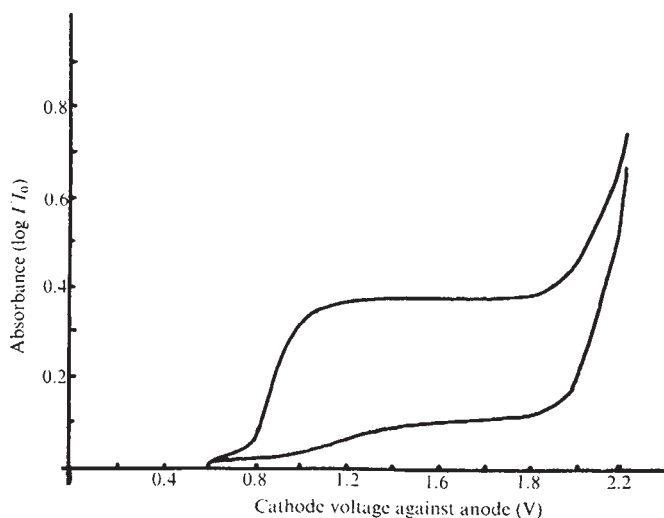


Fig. 1 Variation of absorbance (207 nm) with voltage. Upper curve, 5 p.p.m. Zn^{2+} in 0.03 M K_2SO_4 ; lower curve, 0.03 M K_2SO_4 only.

These observations suggest the transient existence of hydrated free atoms in the solution, close to the cathode surface. It is probable that these are formed by a process of electron tunnelling from the cathode with the formation of hydrated electrons which subsequently reduce the hydrated, or otherwise liganded, cations.

These experiments suggest to us the basis of a potentially useful analytical trace technique of hydrated (solvated) atomic absorption spectroscopy (T. S. West, Fourth International Conference on Atomic Spectroscopy, Toronto, unpublished). The technique may also prove useful for the examination of surface mechanisms in electrodeposition and electrochemistry in general.

A simple ultraviolet/visible spectrophotometer was built from a hydrogen lamp and power supply, a tungsten filament lamp, a monochromator, and a photomultiplier tube with a Branden-

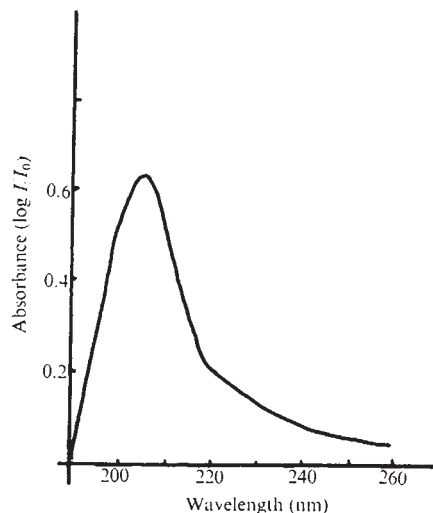


Fig. 2 Variation of absorbance with wavelength; 10 p.p.m. Zn^{2+} in 0.03 M K_2SO_4 at -1.4 V.

burg type 475R e.h.t. power supply. The output was monitored by a chart recorder. A 20-mm silica cell was used, with two platinum plate electrodes inserted. A potentiostatic waveform source was used as a signal generator. The alignment was such that a narrow collimated beam of light passed over the surface of the cathode, with the anode kept well clear of the light path. The cathode could be racked upwards or downwards in the light beam.

Each element was studied by using the highest possible potential difference in order to find a wavelength at which a maximum transient increase in absorbance was obtained. At that wavelength, the optimum electrode pretreatment conditions and potential difference were found, and, using these, the variations of absorbance with wavelength and with concentration of metal cation were obtained.

The stock solutions were diluted to give solutions of (typically) 10 parts per million (p.p.m.) in 0.03 M K_2SO_4 or 0.1 M KCl. A 5 cm³ aliquot was placed in the cell and de-aerated with oxygen-free nitrogen. The gain of the system was adjusted so that zero absorbance was registered, and a potential step (square wave) was imposed on the electrodes so that the electrode in the light path became the cathode. After the change in absorbance had been recorded, the cell was emptied (by suction) and the working electrode was washed with dilute base electrolyte while a high positive potential (up to 5.0 V) was applied—that is, it was cleaned by anodic stripping. If necessary, any surface oxide was reduced by reversing the polarity of the system.

Using this technique we studied the following metal ions: Cu^{2+} , Cd^{2+} , Fe^{2+} , Fe^{3+} , Pb^{2+} , Zn^{2+} , Co^{2+} and Ni^{2+} . On applying a potential difference between the electrodes a transient increase in absorbance was observed. This change in absorbance

has been interpreted as absorption of light by reduced species generated on or near the cathode surface.

A variation of absorbance with voltage was observed, in most cases, after a simple step function had been applied, that is, the potential difference was stepped from 0.0 V to the preset required value. Pulsed square wave functions were also used (both between zero and the required voltage and between positive and negative values) but, in general, these did not increase the magnitude of the absorbance obtained. The magnitude of the potential difference is limited by cathodic evolution of hydrogen (it was limited to about -2.2 V in 0.03 M K_2SO_4). At the absorption maximum, plots of absorbance against potential difference were obtained. These have the appearance of d.c. polarograms; as the potential difference is increased, the absorbance increases until a plateau region occurs. This persists as the applied potential difference is progressively increased until the background electrolyte begins to be reduced, or hydrogen is evolved, at which point a rapid increase in absorbance, resulting from scattering, is observed. Zinc typically has a plateau region of -1.1 to -2.0 V (Fig. 1).

With an oxidised cathode an increased signal was obtained at lower potential differences. At -2.0 V the nature of the pre-treatment had no effect on the magnitude of the absorbance signals observed for any of the cations.

With the background electrolyte alone, no absorbance was observed at wavelengths greater than 210 nm. Below this, a band with a maximum absorbance of about 0.25 (half bandwidth 10 nm) was observed at 197 nm.

Plots of absorbance against wavelength were made at the optimum observed conditions. Generally, these are, not unexpectedly, much broader than gas phase absorption lines and have a single maximum occurring in the ultraviolet. For example, zinc has a maximum at 207 nm and a half bandwidth of 16 nm (Fig. 2). The spectra for the other metals so far examined are broader.

Absorbance against concentration was plotted at the absorption maximum for each metal. Generally, the plots were linear, over a variety of concentration ranges. The zinc calibration was linear between 1 and 12 p.p.m.

The absorption typically reached its maximum value after approximately 30 s and then decayed to the initial value over a period of up to 2 min if the applied potential was maintained. The decay was more rapid if the potential difference reverted to zero at, or just after, the maximum absorbance had been reached.

The maximum absorbance was only maintained if an oscillating voltage function (both positive and negative going) was used at a frequency greater than 0.1 Hz.

Although some metals give rise to more intensely absorbing species, there is a concentration range for each metal so far studied, over which the absorbance is a linear function of concentration. This, together with the possibility of achieving selectivity with a suitable choice of wavelength and voltage, or with selective complexing and masking (as in the preconcentration step in voltammetric stripping analysis), points the way to analytical applications.

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BIOLOGICAL SCIENCES

Comparison of predicted and experimentally determined secondary structure of adenyl kinase

It is generally accepted that the action of a protein cannot be understood until its three-dimensional structure is known. At present, X-ray analysis of protein crystals is the only method of obtaining such structural information. It is to be feared, however, that many important proteins will never give suitable crystals so that one is obliged to consider other approaches to structure elucidation. Renaturation experiments indicated¹⁻⁴ that the three-dimensional structure of many if not all proteins is a unique function of their amino acid sequence. Therefore, in principle one should be able to determine these structures by using only the information contained in the sequence. A first step in this direction is the prediction of secondary structures (α helices, β pleated sheets, β bends) in globular proteins from amino acid sequences. Several prediction schemes have been devised to this end⁵⁻²³. It is the aim of this paper to demonstrate directly the current standing of such methods.

The enzyme adenyl kinase (adenylate kinase, EC 2.7.4.3, molecular weight 21,600) (ref. 24) was taken as a test object. Its amino acid sequence²⁵ was distributed to several groups known to work on prediction schemes. None of these groups knew the three-dimensional structure²⁶ of the protein when giving their prediction; and they were not informed about structural relationships²⁷ between adenyl kinase and other proteins. Therefore, none of the predictions can be biased by previous structural information.

The predicted and the experimentally determined secondary structures in adenyl kinase are compiled in Fig. 1. The experimental data are derived from a 3 Å electron density map. Although such a map is not detailed enough to show hydrogen bonds directly, most of the secondary structures are clearly visible. There are considerable differences between the prediction methods that have been applied. Detailed descriptions of the individual schemes can be found in the references given in the legend of Fig. 1. As shown in the lowest part of Fig. 1 the α helix predictions correspond very well with the experimental data. Two groups found nine, two groups found eight, and one group found seven out of the 10 helices. Only one of these five groups predicted a wrong piece of chain as being helical. The results in Fig. 1 indicate, however, that it is still difficult to find the correct starting and termination point of a helix.

A conventional way of estimating the success of a particular prediction scheme is to compare the prediction with the experiment in every single residue²⁸. Therefore, scores have been made which are listed on the right side of Fig. 1. One group gave an inverse prediction, that is, helix breakers instead of helical residues. To avoid confusion this kind of prediction has not been scored. A 'joint prediction' of α helices was produced by adding predictions A to J at each residue and displaying the result as a histogram. This histogram is in good agreement with the experimental data except for the C-terminal region.

Four groups predicted those residues that are involved in pleated sheet structures and one group assigned sheet-breaking residues. Again, scores have been taken and a joint prediction histogram has been produced. The results show that the three central strands (around residues 12, 91, and 116) of the five-

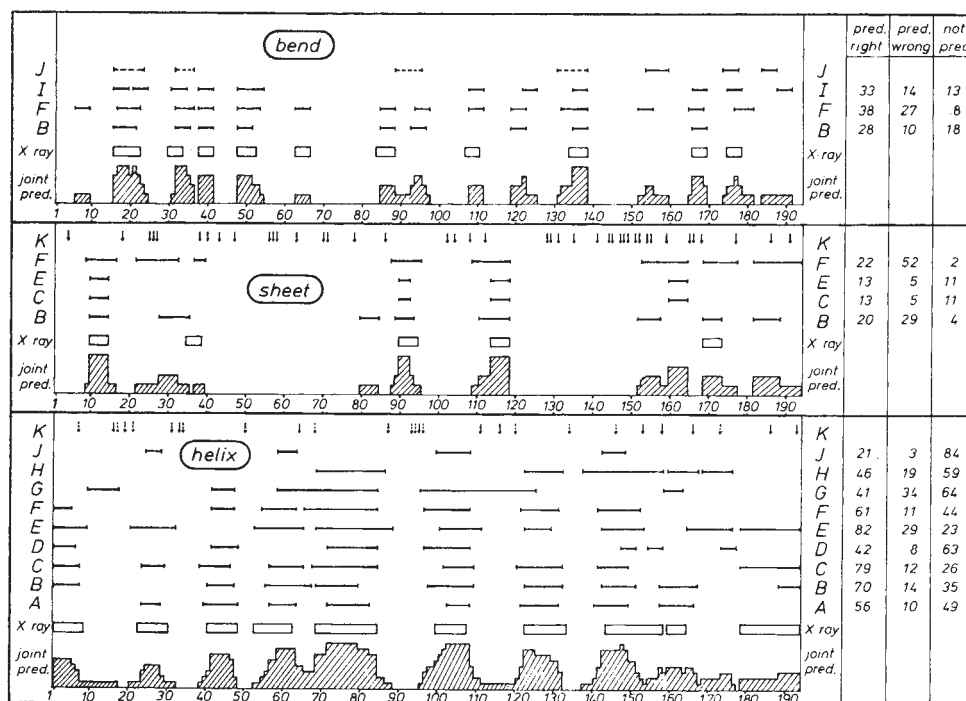


Fig. 1 Comparison of predicted and experimentally determined α helices, strands of β -pleated sheets, and bends in adenyl kinase. The experimental data (X-ray) have been derived from a 3 Å electron density map. At this resolution the exact geometry of bends cannot be evaluated with certainty. Therefore, the experimentally determined bends in Fig. 1 are defined as changes of more than 120° in the overall direction of the polypeptide chain and without reference to any hydrogen bonding scheme. None of the predictions is biased by previous structural information about the enzyme. The predictions A to K have been supplied by A, Barry and Friedman; B, Chou and Fasman^{21,22}; C, Finkelstein and Ptitsyn^{10,11}; D, Levitt and Robson^{14,32}; E, Lim²³; F, Nagano²⁰; G, Burgess and Scheraga⁸; H, Burgess and Scheraga⁹; I, Burgess and Scheraga¹³; J, Burgess and Scheraga¹⁹; K, Kabat and Wu¹⁸. They are based upon prediction schemes that have been described in the corresponding references. (The method applied by Barry and Friedman has not been published yet. It uses an analysis of the distribution of amino acids occurring at both ends of helices to define potential starting and termination points of helices in a sequence. In this method the residues three before and three after, as well as the residue defined as the beginning (or end) of an helix, are considered to be structurally significant. The predictions are based on data for 90 helices from 23 distinct three-dimensional protein structures.) Although predictions are usually made as probability profiles, all groups converted their profiles beforehand to yes or no decisions for each residue by comparing the probabilities with a given threshold value. Thus, every prediction depends on the threshold value applied³². This procedure simplified the comparison appreciably. But concomitantly, it reduced the amount of information contained in any of the predictions. Kabat and Wu predicted helix and sheet breaking residues, which are indicated by vertical arrows. Dashed arrows point to residues that are helix breaking but with a lower probability. Dashed lines in the bend prediction J indicate 'multiple bend regions'. A bend is predicted to be anywhere within such a region. Three joint prediction histograms have been produced by adding predictions A to J for helix, predictions B, C, E, and F for sheet and predictions B, F, I, and J for bends, respectively. Scores of correctly and incorrectly predicted residues as well as residues not predicted are listed on the right side of the figure.

stranded parallel pleated sheet in adenyl kinase have been predicted correctly by all groups. This is not true, however, for the two strands at the edges of this sheet. Scores and histograms indicate that the accuracy of the β -sheet prediction is lower than the accuracy of helix or bend prediction. This might be related to the fact that the sheets are predominantly formed by interactions between residues that are far apart along the polypeptide chain (= nonlocal interactions) whereas helices and bends are caused by interactions between neighbours along the chain (= local interactions). Obviously, local interactions are more easily recognised in an amino acid sequence than nonlocal interactions, because the latter require assumptions about the three-dimensional structure.

The results in the upper part of Fig. 1 as well as the corresponding scores indicate that bends of the polypeptide chain are often successfully predicted. The joint prediction histogram shows that essentially only the bend around residue 64 has been missed. The bends predicted at residues 95 and 122 turned out to be directional changes in the polypeptide chain of only 60° and 90°, which is less than the 120° required in the definition for an experimentally determined bend (see legend of Fig. 1).

As shown in Fig. 1 some of the secondary structures have been predicted consistently whereas others were less readily recognised. The α helices in the middle of the chain and the strands in the centre of the sheet and the bends near to the N terminus were predicted consistently. There is less agreement in the C-terminal region. It is conceivable that consistent predictions are obtained in chain segments where local inter-

actions dominate the formation of secondary structures and that the predictions become more inaccurate whenever non-local interactions predominate. Thus, the prediction problems in the C-terminal region would be understood if during the folding process the C-terminal end is wrapped around a pre-existing molecular core. Nonlocal interactions with this core might force the terminal chain into its final secondary structure.

Adenyl kinase contains a structurally rather independent domain consisting of the three helices between residues 41 and 84. The consistent prediction of these helices indicates that their formation might be dominated by local interactions. Moreover, if local interactions dominate, the helices can form spontaneously so that they may be nucleation points and the domain may be a nucleus^{21,29-31} in the folding process.

In conclusion it seems fair to state that the joint prediction histograms allow the assignment of most of the helices and bends and more than half of the sheet strands. It must be remembered, however, that adenyl kinase is a protein with abundant secondary structures and that joint prediction histograms of other proteins might be less informative. For the same reason the merit or demerit of any prediction scheme should not be judged from its success with this particular protein alone.

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Topological comparison of adenylyl kinase with other proteins

A COMBINATION of amino acid sequence analysis¹ and X-ray analysis has yielded the atomic structure of the enzyme adenylyl kinase² (adenylate kinase, EC 2.7.4.3). No protein of which the structure is known is so closely related to adenylyl kinase that an amino acid sequence comparison³ or an exact geometrical comparison of the structures⁴⁻⁶ can be expected to indicate a relationship. Similarities with other proteins become apparent, however, if one restricts the comparison to topologies, that is, to chain folds without reference to the exact geometry. Since chain folds are particularly well conserved during evolution⁷ such a procedure might reveal distant relationships.

The most important structural feature of adenylyl kinase is its central five-stranded parallel pleated sheet²; therefore, we shall only discuss proteins containing a similar motif. Central parallel sheets with more than three strands are present in subtilisin⁸⁻¹⁰, flavodoxin^{11,12}, and various dehydrogenases¹³⁻¹⁶. The sheets of these proteins are sketched in Fig. 1 according to a proposal of Rossmann (personal communication). The drawings contain no geometrical detail. Only the sheet strands and the approximate path of the connections between these strands (through the upper and lower side of the sheet), that is, only the sheet topology, is given. In such a presentation the topology is fully described by the strand sequence in the sheet and the above-below pattern of the connections. Since there are finite, calculable numbers of possible strand sequences and possible connection patterns, the number of possible topologies (M) is also finite and calculable.

This number can be used to define a quantitative measure of relationship between two structures. Assuming, for instance, each of two proteins contains an n -stranded parallel sheet with identical topology; since such a sheet can exist in M different topologies, the probability that by chance both sheet topologies are identical is $1/M$. Or, in other words, the significance of their relationship, which one might call 'figure of topological relatedness', is $M:1$. We shall now derive such figures within the group of protein structures shown in Fig. 1.

First we have to evaluate the number of possible strand sequences in an n -stranded parallel sheet. This is equal to $n!/2$, the number of permutations of n items reduced by a factor of 2. The halving is necessary because symmetrically related strand sequences $ABCD$ and $DCBA$ can be superimposed by rotation. Second, we take into account that the $n-1$ connections between the strands run either through the upper or the lower side of the sheet, giving rise to 2^{n-1} possible above-below connection patterns. Combining strand sequences and connection patterns we find that there are $M = 2^{n-1} \times n!/2 = 2^{n-2} \times n!$ possible topologies. For simplicity this counting scheme disregards further topological differences which arise whenever connections cross each other as, for example, connections $A-B$ and $B-C$ in subtilisin.

One can use this formula for a comparison within the dehydrogenase family, for example, for a comparison between

Table 1 'Figures of topological relatedness' between protein structures

Compared sheet topologies		No. of strands in common for both sheets	No. of connections in common for both sheets	Uncorrected figure of topological relatedness	Reduction factor	Corrected figure of topological relatedness	Figure of topological relatedness including binding region
Adenyl kinase	Subtilisin	5	3*	480 : 1	1 × 2	240 : 1	2400 : 1
Adenyl kinase	Flavodoxin	4	3	96 : 1	2 × 2	24 : 1	240 : 1
Adenyl kinase	Any dehydrogenase	4	3	96 : 1	2 × 3	16 : 1	160 : 1
Subtilisin	Flavodoxin	5	4	960 : 1	2 × 1	480 : 1	4800 : 1
Subtilisin	Any dehydrogenase	5	4	960 : 1	2 × 2	240 : 1	2400 : 1
Flavodoxin	Any dehydrogenase	5	4	960 : 1	1 × 2	480 : 1	4800 : 1
A dehydrogenase	Another dehydrogenase	6	5	11520 : 1	1 × 1	11520 : 1	115200 : 1

* Adenyl kinase and subtilisin have five strands but only three (and not four) connections in common because connections *B-C* are different. No reduction factor for this disagreement has been applied, however, since connection *B-C* has only slipped around a sheet edge.

lactate dehydrogenase¹³ and liver alcohol dehydrogenase¹⁵. Both structures contain six-stranded parallel sheets with identical topology (Fig. 1), although there exist $M=2^{6-2} \times 6! = 11,520$ possible topologies for such a sheet. Therefore, their "figure of topological relatedness" is 11,520 : 1.

The reasoning can be extended to topologies that are identical in the central strands of the sheet but that do show differences at the sheet edges. The procedure is best described by com-

paring adenyl kinase with flavodoxin. Both structures have a four-stranded sheet (*ACDE*) and three connections (*A-B=A-C*, *C-D*, *D-E*) in common. This yields an "uncorrected figure of topological relatedness" of $2^{4-2} \times 4! : 1$ or 96:1. For the comparison, however, one strand in each protein has been discarded. When selecting these strands an arbitrary choice of one out of two possibilities in each protein (left or right edge) has been made. Since such a choice reduces the significance, a reduction factor of $2 \times 2 = 4$ was applied to the "uncorrected figure of topological relatedness", yielding a "corrected figure of topological relatedness" of 24:1 (see Table 1). An analogous procedure has been followed when comparing other pairs of structures (Table 1).

The structural relationship within the dehydrogenase family is not only indicated by correspondence of sheet topologies but also by correspondence of nucleotide binding positions¹⁶. As shown in Fig. 1, the other proteins contain similar sites at similar positions relative to the sheet. Therefore, we shall include this kind of information about relationship into the proposed quantitative measure.

For sake of simplicity we do not distinguish between different kinds of sites but merely refer to them as being a "binding region". In the view given in Fig. 1, for all proteins this binding region is located in front of the carbonyl ends of the central sheet strands, that is, above the plane of the paper. In order to use the same reasoning as before, we estimate how many positions of such a binding region we would have accepted as being different. For instance, we would have been able to distinguish between left side, centre, and right side of the sheet as well as between above, in front, in the rear, and below the sheet, that is, we would have differentiated between about 10 positions.

Since all proteins show the same binding region position out of these roughly estimated 10 possibilities, there is an additional significance of their relationship of 10:1. Thus, the 'corrected figure of topological relatedness' (Table 1) may be multiplied by a factor of 10, yielding the overall 'figure of topological relatedness including binding region' between the structures (Table 1). If a closer correspondence of the binding regions could be established, there would be more than 10 distinguishable positions and therefore a larger additional factor.

The results in Table 1 show that adenyl kinase is rather closely related to subtilisin and less closely to flavodoxin and to the dehydrogenase family. This corresponds to the strand sequence reversal *BC* compared with *CB* between adenyl kinase and subtilisin on the one hand and the dehydrogenases on the other. A relationship between the dehydrogenases, flavodoxin and subtilisin is clearly indicated.

These relationships point to either divergent or convergent evolution. Convergent evolution would be expected if the resulting topology is energetically favoured or if the topology is favoured in the dynamical folding process¹⁸ of the polypeptide chain. But this does not seem very likely. In the case of divergent evolution, however, we have to postulate a common ancestor for enzymes as distant from each other as the

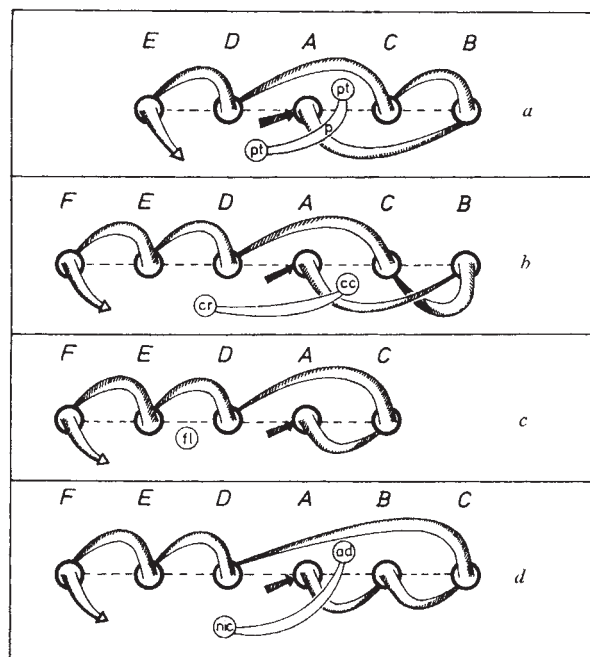


Fig. 1 Topologies of proteins containing a parallel sheet. *a*, Adenyl kinase²; *b*, subtilisin⁸⁻¹⁰; *c*, flavodoxin^{11,12}; *d*, NAD-binding globules (domains) of lactate dehydrogenase¹³, s-malate dehydrogenase¹⁴, liver alcohol dehydrogenase¹⁵, and D-glyceraldehyde-3-phosphate dehydrogenase¹⁶; pt, hydrophobic pockets, that is, presumed binding sites for adenine²; p, phosphate position in the catalytic centre of adenylate kinase; cr, hydrophobic crevice, that is, presumed binding site for aromatic or apolar side chain¹⁰; cc, catalytic centre; fl, FMN; nic-ad, nicotinamide and adenine moieties of the dinucleotide NAD. In the case of adenyl kinase the five heavy circles are the five strands in the sheet. (The drawing corresponds to the linear diagram given in Fig. 3 of the adenyl kinase structure description², if one views this figure from the top along the strands in the sheet, that is, along the plane of the paper, to the bottom.) The carbonyl ends of these strands point toward the viewer. The arrows indicate the direction of the polypeptide chain³, that is strands *ABCDE* in the sheet are ordered alphabetically along the course of the chain. The sheet topologies of the other proteins have been drawn correspondingly. The topologies of the NAD-binding globules of the four dehydrogenases are identical¹⁶. The same topology might be present in horse muscle phosphoglycerate kinase¹⁷. The location of substrates (pt, cr), cosubstrates (fl, nic-ad), and catalytic centres (cc, p) are all in front of the carbonyl ends of the central strands in the sheet, that is above the plane of the paper.

bacterial extracellular protease subtilisin and the mammalian intracellular adenyl kinase.

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Stimulatory capacity of human T and B lymphocytes in the mixed leukocyte culture

LYMPHOCYTES can be separated into thymus-dependent (T) and thymus-independent (B) cells on the basis of ontogeny, surface membrane differentiation markers, and function. Thus, some functions can be attributed solely to T or B cells, whereas others require T/B cell cooperation^{1,2}. When two populations of allogeneic lymphoid cells are cultured together in the mixed leukocyte culture assay (MLC), cellular interaction leads to blastic transformation and subsequent proliferation of a portion of the cultured cells. This proliferative response originates in T lymphocytes^{3–5,30,31}. But secondary T cell-mediated B cell proliferation may in some species contribute to the overall proliferative response of T and B cells^{6–9}. The question then arises: which cell type acts as stimulator in MLC? Is the stimulus to proliferate equally transferred by T and B cells, or are the antigenic determinants responsible for MLC activation represented exclusively or preferentially on the surface of only T or B lymphocytes? Studies in mice^{9–11} indicated that T and B cells possess equal stimulatory capacities. However, in other reports the stimulatory capacity in mouse MLC was almost

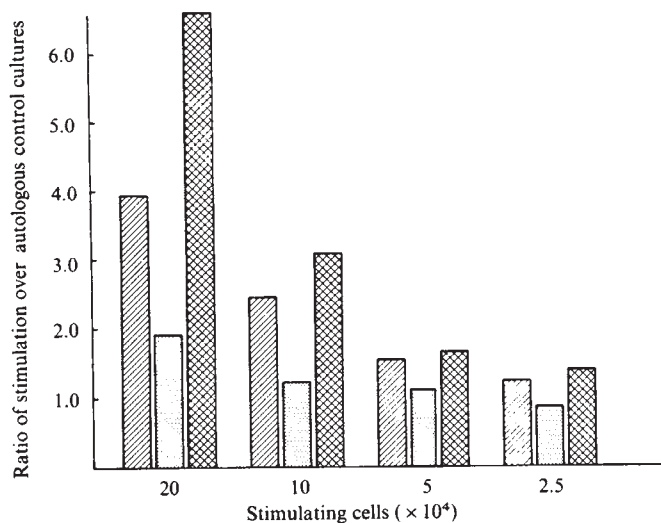


Fig. 1 Stimulatory capacity of unseparated T and B peripheral blood lymphocytes in human MLC. Lymphocytes (2×10^5) were stimulated with decreasing numbers of irradiated allogeneic cells. Ordinate: ratio of stimulation (^3H -TdR uptake in allogeneic cultures over ^3H -TdR uptake in corresponding autologous cultures), as calculated from the means of triplicate cultures. Abscissa: number of stimulating cells. Left bar, Unseparated lymphocytes; Centre bar, T lymphocytes; Right bar, B lymphocytes.

exclusively attributed to B lymphocytes (refs 3 and 12, and personal communication from D. H. Sachs).

We have attempted to answer this same question for human peripheral blood lymphocytes and report here that the proliferative response in human MLC is largely the result of stimulation by B cell-bound alloantigens.

Cells responding in MLC were prepared from heparinised venous blood by centrifugation over a Ficoll-Hypaque gradient¹³. This preparation regularly contained more than 90% lymphocytes, the rest being monocytes and an occasional granulocyte.

Rosette formation of human T lymphocytes with unsensitised sheep erythrocytes (SRBC)¹⁴ was utilised to separate peripheral blood lymphocytes, used as stimulating cells in MLC, into rosetting (T) and nonrosetting (B) cells. To remove monocytes, which stimulate, although weakly, in MLC¹⁵, heparinised whole blood was incubated with sterile carbonyl iron powder for 60–90 min at 37° C with continuous agitation. Subsequent centrifugation over a Ficoll-Hypaque gradient yielded lymphocyte preparations containing less than 0.1% monocytes. Using a modification of the method of Wybran *et al.*¹⁶, these lymphocytes were rosetted with neuraminidase-pretreated SRBC (N-SRBC) which bind more firmly to human T lymphocytes than do untreated SRBC^{17,18}. Samples of 0.25 ml lymphocytes in Hanks balanced salt solution (HBSS) (10^7 ml^{-1}), 0.25 ml of foetal calf serum (absorbed by SRBC; Microbiological Associates) and 0.5 ml of N-SRBC (10^8 ml^{-1}) were added to plastic test tubes, centrifuged for 8 min at 200g, and further incubated at room temperature for 60 min. The gently resuspended pellets of all tubes (containing rosetted and nonrosetted lymphocytes) were pooled, and centrifuged over a Ficoll-Hypaque gradient (30 min at 400g); rosetting cells formed the pellet and nonrosetting cells remained at the interface between the layers. The pellet lymphocytes (termed T cells here) were washed twice in HBSS, and resuspended in culture medium (see below) to the concentration needed. No efforts were made to lyse or remove the N-SRBC so as to manipulate the lymphocytes as little as possible: orienting experiments showed that the capacity of lymphocytes to stimulate or respond in MLC was unaltered by addition of similar numbers of N-SRBC. The interface cells were washed, rosetted and again centrifuged over a Ficoll-Hypaque layer, to remove residual rosetting cells. The inter-

face cells thus obtained will be termed B cells here. This procedure effectively separated T and B cells: preparations of B cells contained less than 1% rosetting lymphocytes; and less than 2% of the T lymphocytes carried detectable amounts of surface immunoglobulins, as determined by direct immunofluorescence techniques¹⁷.

MLC (1 ml each) were set up in duplicate or triplicate with 400 ml Eagle's minimal essential medium (MEM) supplemented with 75 ml of decomplexed pooled human serum, 10 ml of L-glutamine (200 mM), 10 ml of a mixture of streptomycin and penicillin (5,000 U ml⁻¹ each), and 5 ml of nonessential amino acids (Flow Laboratories). To obtain one-way stimulation in MLC, stimulating allogeneic lymphocytes were irradiated (2,500 rad; 1,660 rad min⁻¹ from a caesium source) immediately before addition in various numbers to 2×10^5 responding cells. Responsiveness in these one-way MLCs was attributed to the nonirradiated cells, whereas stimulation was considered to be a function of the irradiated cell populations. Cultures were maintained in a humidified 5% CO₂-air atmosphere at 37° C for 7 d. Lymphocyte stimulation was assessed from the uptake of tritiated thymidine (³H-TdR) into DNA during the last 4 h in culture; cells were processed as described previously¹⁹.

Decreasing numbers of stimulating unseparated, T or B lymphocytes were added to 2×10^5 responding cells. Figure 1 demonstrates that B cells stimulated three times more vigorously than T lymphocytes using a ratio of responder:stimulator cells of 1:1. When compared with unseparated lymphocytes, T cells possessed significantly lower and B cells higher stimulatory capacity ($P < 0.001$). At lower responder:stimulator ratios, these differences were less pronounced.

Mixtures with different proportions of T and B cells were prepared, and used for stimulation of allogeneic lymphocytes. Figure 2 shows the proliferative response at three different concentrations of stimulating cells. Again, the stimulatory capacity of B cells was more than three times that of T cells at a responder:stimulator ratio of 1:1. Increasing percentages of T cells among stimulating cells led to decreasing stimulation. Similar, but less striking findings were obtained with lower numbers of stimulating cells. Could the stimulatory capacity of T cells be altered by formation of rosettes? Adherence of N-SRBC may expose previously masked T cell antigens for MLC stimulation; alternatively, T cell alloantigens may be removed or altered so that the T cells' stimulatory capacity is reduced or lost. The data presented here refute these objections. The stimulatory capacity of mixed T and B cells, using a T:B cell ratio of 2:1, was close to that of unseparated lymphocytes. Since there is a T:B cell ratio of approximately 2:1 in unseparated normal peripheral blood lymphocytes^{14,17}, it can be concluded that T cells possess their original MLC-stimulating capacity after rosette formation. Data in Fig. 2 further suggest that B and T cells contribute independently and additively to the stimulatory capacity of unseparated lymphocytes. To test whether T cells could suppress the stimulatory capacity of B cells, a constant number of stimulating B cells (2×10^5) and increasing numbers of stimulating T cells (0 to 2×10^5) were added to 2×10^5 responding lymphocytes. The proliferative lymphocyte response increased with increasing numbers of stimulating T cells; this observation excluded T cell suppression of the stimulatory capacity of B cells.

These results demonstrate that the major stimulus in human MLCs has to be attributed to B cell-bound alloantigens, and that human T cells possess only weak MLC-stimulatory capacity. The strength of the stimulatory capacity of the T cell preparations renders it unlikely that the few residual contaminating B cells were solely responsible for this stimulation; true T cell-bound stimulatory capacity seems to exist in man.

We propose the following hypothesis to explain the strong

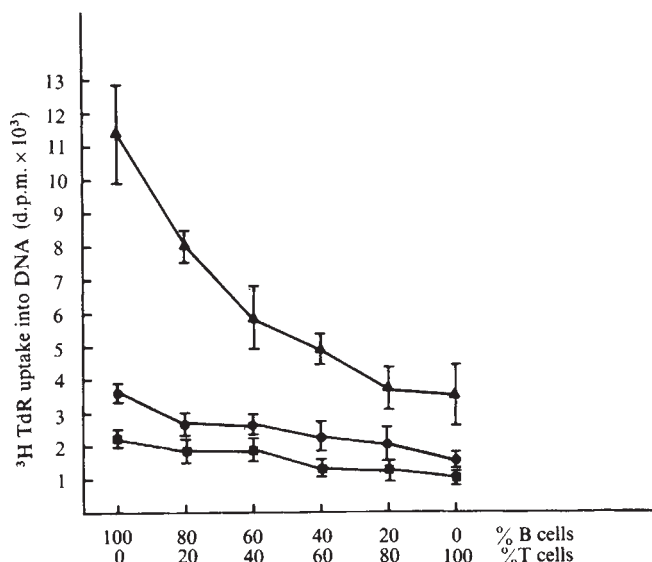


Fig. 2 Stimulatory capacity of human T and B lymphocytes in MLC. Responding cells (2×10^5) were stimulated with mixtures of allogeneic T and B cells at three different concentrations. Abscissa: percentages of T and B cells in the stimulating cell mixtures. Ordinate: ³H-TdR uptake into DNA (d.p.m.); indicated are the means $\pm$ one s.d. of triplicate cultures. Δ , 2×10^5 stimulating cells; $\bullet$, 5×10^4 stimulating cells; $\blacksquare$, 2×10^4 stimulating cells.

B cell and weak T cell stimulatory capacity in human MLC. Serologically defined (SD) alloantigens (H-2 in the mouse, HL-A in man) were originally thought to be responsible for MLC activation²⁰. From studies in intra-H2 recombinant mouse strains²¹ and in siblings with a crossing over event within the major histocompatibility complex (MHC)^{20,22,23}, however, it is generally accepted that genes closely linked to, but genetically separable from, the two SD loci code for MLC activation (MLC loci, LD loci). Since genetic differences in the Ir region are required for strong MLC stimulation between congenic mouse strains^{21,24}, Ir-linked genes have been implicated as coding for MLC-activating gene products²⁵. A B-cell-specific alloantigen, coded by Ir-linked genes²⁶, has been suggested to be responsible for the strong one-way MLC activation observed between two recombinant mouse strains^{12,27}. But at least one other MLC locus exists within the MHC of mice, coding for a weakly stimulating alloantigenic system²⁴.

Two MLC loci have also been suggested in man, one coding for a strong and the other coding for a weak MLC-stimulating alloantigenic system^{28,29}. It is tempting to speculate that, in further analogy to the findings in the mouse, the differences in the MLC-stimulatory capacity between human T and B cells are due to different expression of MHC-determined alloantigenic gene products on T and B cells: the genetic locus responsible for strong MLC stimulation may code for B-cell-specific alloantigens, whereas the gene products of the second locus, coding for weak MLC-stimulating alloantigens, may be represented on T (and B?) cells. Further studies on the stimulatory capacity of T and B cells in siblings with a recombinational event in the MHC may substantiate this hypothesis.

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Successful construction of chimaeric rabbit

CHIMAERIC mice can be made by aggregation of zona-free cleavage stage embryos, and these animals have proved useful in analysing various aspects of development and differentiation. Animals of this type are particularly suited to test models which propose that genetic information is transferred between cells, for example during antibody formation. It has been reported that RNA extracted from immunised rabbits can induce cells homozygous for one *ii* immunoglobulin allotype to synthesise antibody molecules carrying a foreign allotype characteristic of the animals from which the RNA had been extracted¹⁻³. Although chimaeric mice have been used to search for gene transfer during the normal *in vivo* response to antigen, these results have not proved conclusive⁴.

The concept of gene transfer in the immune response

Table 1 The immunoglobulin allotypic markers in the parental rabbits and their offspring

	Coat Colour	Heavy chain		Light chain	
		a1	a3	b4	b5
Parental colony 1	White	+		+	
2	Black		+	+	+
Offspring 1	White	+		+	
2	Chimaeric	+	+	+	+

The pattern of allotypic markers seen in the offspring has been consistently observed over an 8 month period. The threshold of detection of the 5b allotypic marker was less than 1/625 of the concentration in normal serum.

originated from work in rabbits and, in addition, the distribution of the allotypic markers in different parts of the immunoglobulin molecule⁵ make the rabbit the ideal species to answer the problem.

It is unlikely that chimaeras formed by aggregation of zona-free cleavage stage embryos will be successful in the rabbit as it has been shown that zona-free blastocysts fail to implant (C. E. Adams, unpublished). Chimaeric mice can be constructed, however, by the injection of isolated inner cell mass cells or the insertion of inner cell masses into the complete blastocyst still surrounded by a zona pellucida⁶. Here we report the successful application of this method to the rabbit.

Inner cell masses were dissected from 4-d-old blastocysts obtained from black rabbits bearing the heavy chain allotype a3 and the light chain allotypes b4 and b5, and individual inner cell masses were inserted into blastocysts, also 4 d old, obtained from white rabbits carrying the heavy chain allotype a1 and the light chain allotype b4. The blastocysts were allowed to repair during a brief incubation in culture (up to 2 h) and then returned to the uterus of pseudopregnant white rabbits which carried the a1 and b4 allotypes. Pseudopregnancy was induced 4 d before the transfer of the blastocysts.

Five blastocysts were transferred to each of two rabbits. Two offspring were obtained from one of these rabbits, and none from the other. One of the offspring was an albino rabbit, but the other was a chimaera with mixed coat colour (Fig. 1).

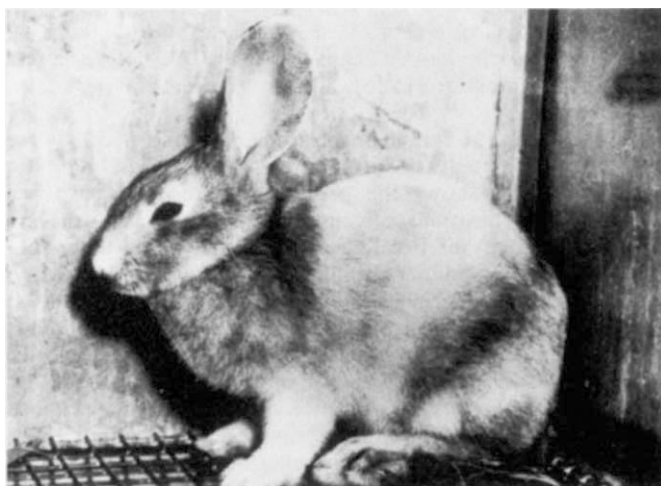


Fig. 1 Chimaeric rabbit, 37 d old.

Allotype analysis was carried out on the serum obtained from the two rabbits and the results are shown in Table 1. The albino rabbit contained only the a1 and b4 allotypes but the chimaeric rabbit contained the a1, a3, b4 and b5 allotypes. Further chimaeric animals with this gene combination are being produced and we are at present collecting reagents to look for gene transfer in the existing chimaera. Phenotypically the chimaeric animal is female and has produced a litter of nine albino rabbits following mating with a New Zealand white buck. Ten metaphases from circulating blood have proved to be XX. We hope animals of this type will provide an unambiguous answer to the problem of gene transfer in the immune response.

We thank S. C. Barton, K. Day and B. Burgess for their help and the Medical Research Council and Ford Foundation for support. The typing antisera were a gift from Dr Kelus.

Note added in proof: A further chimaeric rabbit has been obtained since this report was submitted for publication.

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Is chiasma determination sequential?

MOST cytological studies of chiasma distribution in higher plants and animals are limited to qualitative statements on the pattern of chiasma distribution along bivalents. A few quantitative studies exist, however, based on detailed measurements of chiasma positions along the extended diplotene bivalents of grasshopper and locust spermatocytes¹⁻³. These studies differ in certain respects but agree in concluding that chiasma formation or at least chiasma determination (assuming that the determination of sites for exchange is separable from exchange itself⁴) occurs sequentially along bivalents. To this extent these studies largely endorse the view originally proposed by Mather^{5,6} which was inferred from the property of chiasma interference and from observations on the frequencies of crossing over in different chromosome regions of *Drosophila melanogaster*.

The cumulative frequency distributions of chiasmata along meiotic bivalents of the locust, *Schistocerca gregaria*^{1,3} and of four species of British grasshopper², demonstrate clearly that chiasmata do not occur with equal frequencies in all chromosome regions. Certain regions, notably the telomeric, distal, ends of both metacentric and telocentric bivalents form chiasmata much more frequently than expected if chiasma distribution were uniform. This distinct peak of distally located chiasmata in all the species included in the studies quoted above has been widely interpreted as evidence that chiasma determination follows a sequential pattern starting at the telomere and extending to other bivalent regions. There is also a distinct tendency, especially in *Schistocerca gregaria*^{1,3}, for bivalents which form only one chiasma (monochiasmate bivalents) to have that chiasma localised distally. This tendency has also been held to demonstrate a sequence in chiasma determination since, for example, it is reasoned that the first-formed chiasma is the

least likely to be lost when chiasma frequency is reduced. There is also an implicit suggestion, based on this observation, of nonindependence between chiasmata formed at the telomere and those formed elsewhere, such that chiasma formation in nontelomeric regions is conditional upon previous telomeric chiasma formation—which again implies a sequence.

A critical examination of these claims shows that the case for a sequential mechanism of chiasma determination is not proved. The frequency peaks of chiasmata in certain chromosome regions, notably at the telomeres, merely denote that these regions enjoy a high probability of chiasma formation and this evidence does not necessarily imply that these chiasmata are the first formed. Likewise, the strict distal localisation of chiasmata in monochiasmate bivalents need not imply a sequence originating at the telomere. Indeed, the argument that nontelomeric chiasmata are conditional upon previous telomeric chiasma formation can be shown to contain a logical fallacy. The spurious impression of dependence is caused by the almost invariable occurrence of one chiasma in the telomeric region.

Observations on the frequency distribution of chiasmata along the bivalents of grasshoppers and locusts therefore make possible, but do not require, a sequential mechanism of chiasma determination. Other models of chiasma determination, including nonsequential models, are not excluded by this evidence and are equally valid if the evidence is taken at its face value.

An inherent feature of all sequential models of chiasma control is the necessary assumption that the control of the chiasma formed first differs fundamentally from the control of subsequent chiasmata. The principal controlling factor identified in these sequential models is chiasma interference but it is evident that this can only influence the position of the second and any subsequent chiasmata, and it follows that the position of the chiasma formed first must be determined by a quite separate control, although the actual mechanism of exchange initiation may be the same for the first chiasma as for subsequent chiasmata. The chiasma properties of a recently described meiotic mutant of rye raise some doubts concerning this distinction between various categories of chiasmata. This mutant genotype was isolated among the segregants from an interspecific cross^{7,8}, and is defective in the control of chiasma distribution⁹. Wild-type rye is characterised by pronounced distal chiasma localisation, so that bivalent arms forming a single chiasma normally have that chiasma localised in a distal, telomeric, position. Additional chiasmata, when they occur, necessarily form in more interstitial locations. According to sequential models of chiasma determination, the distal chiasmata should be regarded as the first formed and as such under a separate control from other, interstitial, chiasmata whose positions should be governed by chiasma interference.

In the distributional mutant of rye, all chiasmata are distributed in an apparently uncontrolled manner. Frequent instances of interstitial and even proximal chiasmata occur even in monochiasmate bivalents⁸, indicating a breakdown of localisation and a more random distribution pattern along bivalents than is found in wild-type rye. In addition, chiasma interference seems to be greatly reduced in intensity, or even absent, in this mutant⁸ (as judged from the Poissonian nature of the between-bivalent frequency distribution of chiasmata¹⁰). These findings indicate that a single control apparently governs the positions of all chiasmata and therefore determines both chiasma interference and chiasma localisation in rye, otherwise one might expect a differential response of these two factors to a change in the controlling mechanism. An extension of this comparison to include a large collection of diverse rye genotypes⁹ indicates that this correlated response is general and widespread (Fig. 1). Furthermore, there is evidence of similar correlated effects in certain meiotic mutants of *Drosophila melanogaster* which show both increased coincidence values (that is, reduced interference) and also nonuniform reductions in crossover frequencies along chromosomes (that is, reduced localisation)¹¹.

There is considerable evidence, therefore, for a single genetic control which governs the positions of all the chiasmata formed

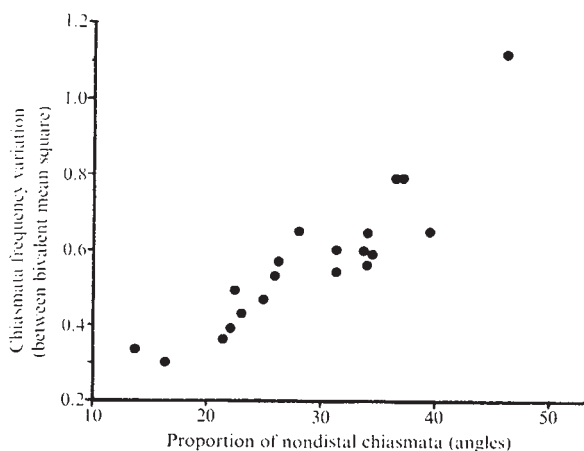


Fig. 1 The relationship of between-bivalent chiasma frequency variation (between-bivalent mean square) and chiasma positional distribution along bivalents (proportion of non-distal chiasmata) for a range of rye genotypes (from Jones⁹). The between-bivalent mean square can be regarded as a rough measure of the strength of chiasma interference¹⁰ and the proportion of nondistal chiasmata is a measure of the degree of chiasma localisation. These variables are clearly correlated ($r = 0.9$; $P < 0.001$) thus indicating that these diverse aspects of chiasma variation are controlled jointly.

in a bivalent, irrespective of their relative positions. The distinction between the chiasmata formed first and subsequent chiasmata, indicated by sequential models of chiasma determination, is difficult to reconcile with this conclusion. Proponents of sequential models could still argue that separate position-determining controls for the first and subsequent chiasmata have a factor in common, but as yet there is no evidence (for example, from the analysis of mutants) for the existence of unique factors associated with these hypothetical separate controls. Models based on nonsequential determination of all chiasmata are therefore at least as acceptable on present evidence and merely require that chiasma interference and other factors such as chiasma localisation be regarded as manifestations or consequences of control rather than control mechanisms in their own right.

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Decreased antibody formation in mice exposed to lead

SEVERAL environmental contaminants have been demonstrated to be synergistic to infectious agents. Polychlorinated biphenyls¹, arsenicals², cobalt sulphate³ and sulphur dioxide⁴ increased the mortality of animals infected with viral agents. Lead nitrate enhanced the mortality of mice to *Salmonella typhimurium*⁵. Some of these compounds are apparently immunosuppressive as circulating antibody titres to infectious agents from animals exposed to lead, cadmium, mercury⁶, DDT (ref. 7) and polychlorinated biphenyls⁸ were significantly lower than those from the control animals. This study was undertaken to determine if the decreased circulating antibody in animals that were exposed to lead was a result of a decrease in the number of cells producing antibody.

Swiss Webster mice 28 d old were given lead orally (1,375, 137.5 or 13.75 p.p.m.) as lead acetate in deionised water for 56 d. The controls were given deionised water. There were 120 mice in each group. The diet fed to all the mice was contaminated with 1.12 p.p.m. lead⁹.

After 56 d, all the mice were inoculated intraperitoneally with 0.2 ml. of a 2% suspension of sheep red blood cells (SRBC). Ten mice in each group were killed on days 3–7 to measure primary immune response (19S or IgM antibody) and on days 8–14 for the secondary response (7S or IgG antibody) after a second inoculation of SRBC on day 7. An additional group of mice were inoculated with SRBC while they remained on 137.5 p.p.m. lead. The spleen from each mouse was immediately removed, cut into pieces, and forced through a nylon mesh into sterile medium 199. The spleen cell suspension was counted in a haemocytometer by the trypan blue exclusion method and diluted to 1×10^7 spleen cells ml⁻¹. The number of plaque forming cells (PFC) was measured according to a modification of Cunningham and Szenberg¹⁰. Three pieces of double-sided tape $\frac{1}{2}$ inch wide were laid across

a clean microscope slide (75 × 25 mm) dividing it into two equal areas. Two clean coverslips (22 mm square) were placed on the tape such that two edges of each coverslip were firmly attached to the tape and formed two shallow chambers. A mixture containing 88% spleen cells (1×10^7), 2% SRBC, 5% complement and 5% medium 199 was delivered by a syringe into the two chambers. These were sealed with vaseline and incubated at room temperature for 2 h. The plaques were counted using a light microscope. The number of plaques per million spleen cells was calculated from the number of plaques observed per μ l of the initial mixture used.

The same procedure was used to assay the secondary response (animals killed on days 8–14). Medium 199 was replaced in the mixture by developing antiserum (Microbiological Associates, Bethesda, Maryland No. 52-122). The number of plaques per million spleen cells was corrected by determining the sensitivity of the developing antiserum and by determining the number of plaques that occurred in spleen suspensions from non-antigen (SRBC) stimulated mice.

The mice were bled every 2 weeks to observe the erythrocytes for basophilic stippling and to determine packed cell volume. Serum was collected before death for haemolysin titration. One kidney was collected at necropsy and stored at -70°C for lead analysis. The other kidney was fixed in 10% buffered formalin for microscopic examination.

There was a reduction in the number of spleen cells producing 19S antibody in each dose of lead exposed mice (Fig. 1) but the decrease was most remarkable in the mice that received 1,375 p.p.m. lead. The peak response was on day 5 for all groups of mice but those given 13.75 and 1,375 p.p.m. lead also had a lag in their response as only a few plaques developed on day 4. The secondary immune response was markedly affected in all of the lead exposed animals. Even those mice that received the lowest lead dose (13.75 p.p.m.) had a significant decrease in the number of 7S plaque-forming cells. The control mice had 500 plaque-forming spleen cells on day 2 and the mice with 13.75 p.p.m. lead had 158 plaques per million spleen cells (Fig. 2). Furthermore, the mice on 137.5 and 1,375 p.p.m. lead had a lower response and a delay of 1 d in their peak response. They also remained much lower throughout the 7 d test period than did the controls. The mice that remained on 137.5 p.p.m.

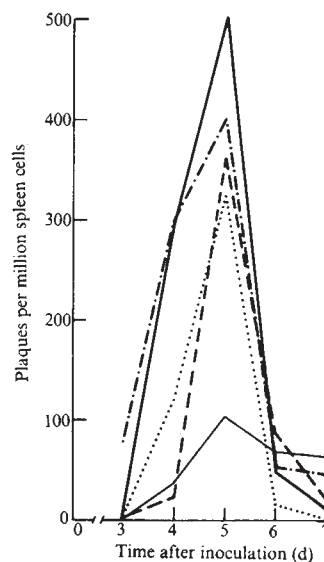


Fig. 1 Primary immune response. The largest lead dose (1,375 p.p.m.) administered to the mice resulted in a five-fold decrease in antibody forming cells as compared to the controls. A 1 d lag in the antibody response occurred in two lead groups (13.75 and 1,375 p.p.m.). There was a highly significant difference in the means at days 3, 4 and 5 as determined by the analysis of variance. —, Control; ---, 13.75 p.p.m. Pb; ···, 137.5 p.p.m. Pb; — · —, 1,375 p.p.m. Pb.

lead throughout the study had fewer plaques, particularly IgG, than did those in which lead was discontinued at the time of inoculation of antigen (Figs 1 and 2).

All of the mice that received 1,375 p.p.m. lead exhibited basophilic stippling of the erythrocytes at 2 weeks of exposure. Basophilic stippling occurred in some of the mice that received 137.5 and 13.75 p.p.m. lead at 4 weeks and in all of those mice at 8 weeks. A few of the control mice had basophilic stippling at 6 and 8 weeks as the diet contained 1.12 p.p.m. lead. The packed cell volume was not significantly different for the various groups throughout the experiment.

As the sera collected at the time of death were haemolysed from the lead the mice received, the haemolysis titrations were quite variable and were considered inaccurate for comparing the circulating antibody titres with the number of plaque-forming cells.

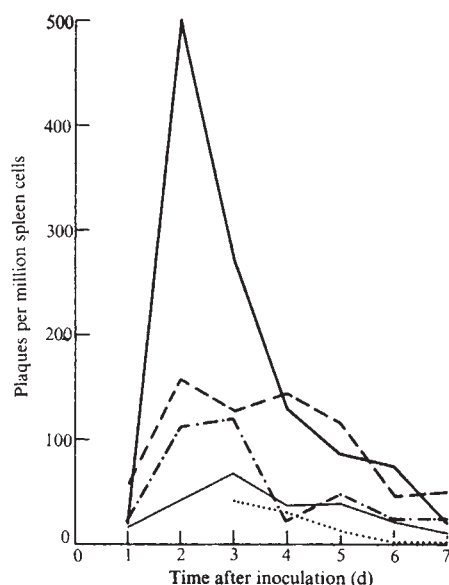


Fig. 2 Secondary immune response. Day 1 represents that day after the second inoculation of the antigen (SRBC), or actually day 8 of the experiment. All lead exposed mice had a highly significant decrease in antibody forming cells as compared to the controls. The two larger doses of lead (13.75 and 1,375 p.p.m.) produced a delay of 1 d in their peak response. The antibody production was the lowest in those mice that were on lead continuously until death. There was a significant difference in the means at day 3 and 4 and a highly significant difference at days 1, 2 and 7 as determined by the analysis of variance. —, Control; ---, 13.75 p.p.m. Pb; ···, 137.5 p.p.m. Pb; — · —, 1,375 p.p.m. Pb (continuous); —, 1,375 p.p.m. Pb.

Histopathology disclosed a marked necrosis of the tubular epithelial cells in the renal cortex, especially near the cortical medullary junction, of the mice that were exposed to 1,375 p.p.m. lead. Many intranuclear inclusion bodies (stained with haematoxylin and eosin (H & E)) were in the renal epithelial cells of these mice for the 14 d period. The renal necrosis was not as extensive and there were fewer intranuclear inclusions in the mice that received 137.5 p.p.m. lead. Also, at day 14, the renal necrosis was not as severe and only an occasional inclusion could be found. There was moderate necrosis of the renal epithelial cells and an occasional intranuclear inclusion until day 5 in the mice that had been given 13.75 p.p.m. lead. From days 6–9, a narrow zone of mild degeneration and necrosis of the epithelial cells was in the cortex adjacent to the cortical medullary junction. Intranuclear inclusions were not observed by H & E stain. From days 10–14, the kidneys appeared to be normal and did not differ from the control kidneys. The Ziehl-Neelsen stain was used to confirm the acid-fast inclusion body that contained lead.

Table 1 Renal concentration of lead from mice that were exposed to lead for 56 days in the drinking water. The kidneys were collected 3 and 14 days after lead was removed from the water

Drinking Water p.p.m. lead	Mean p.p.m. of renal lead*	
	Day 3	Day 14
1,375	16.4 ± 1.97	11.0 ± 0.96
137.5	9.3 ± 0.62	6.7 ± 0.92
13.75	1.0 ± 0.13	0.9 ± 0.09
No lead (control)	0.6 ± 0.09	0.4 ± 0.05

*Wet weight.

The kidneys were analysed for lead content by atomic absorption spectrophotometry using a micro-carbon furnace. The renal concentrations of lead at 3 and 14 d after exposure are listed in Table 1.

It has been shown¹¹ that lead acetate impairs hepatic phagocytic activity in rats but does not alter the primary or secondary immune response. More recently, however, lead acetate markedly reduced the serum antibody titre of pseudorabies virus in rabbits⁶. In the present study, lead acetate markedly decreased the number of cells forming antibody to SRBC suggesting that the decrease in circulating antibody is due to a decrease in antibody formation. Other environmental contaminants such as polychlorinated biphenyls⁸, cadmium⁶, mercury⁶, DDT (ref. 7), and sulphur dioxide¹² have also resulted in reduction of circulating antibody in animals.

Mice given sulphur dioxide for 135 d had an enhancement of antibody production but at 192 d the response was a suppression of antibody¹². It was suggested that high concentrations of sulphur dioxide served as an adjuvant. Lead, however, is immunosuppressive and the larger amount accumulated results in a smaller quantity of antibody synthesised. The group of animals that continued to receive lead until killed had a very slight IgG antibody response and would be most susceptible to infectious agents.

The smallest dose in this study was 13.75 p.p.m. lead in the drinking water, so the mice were ingesting about 0.069 mg of lead d⁻¹. As approximately 10% of ingested lead is absorbed¹³, each mouse was actually receiving about 0.0069 mg of lead into its system each day. A significant decrease in antibody forming cells, particularly 7S, occurred at this dose. The adult human normally ingests 0.3 mg of lead d⁻¹ and 2 mg d⁻¹ can produce toxicity¹⁴.

The intranuclear inclusion bodies in the kidneys are composed of lead and protein¹⁵. They are an acute manifestation of lead poisoning and can occur as early as 24 h after a single intraperitoneal injection¹⁶. In the present study, mice that received 13.75 p.p.m. lead had intranuclear inclusions up to 5 d after removal from lead, but they were not identified thereafter by H & E staining. Also, the cellular degeneration and necrosis in the renal cortex from this group of mice was not as severe at day 6 and could not be recognised by day 11; indicating that there was apparently rapid removal of lead from the kidney after discontinuing administration of lead as well as regeneration of the damaged tubular epithelium.

We have shown that chronic exposure to lead produces a significant decrease in antibody synthesis, particularly IgG, indicating that the memory cell is involved. These results also indicate that the reduced antibody synthesis is responsible for the increased mortality from bacterial and viral diseases in animals that are chronically exposed to lead.

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Dissociation between effects of nerve growth factor on tyrosine hydrolase and tubulin synthesis in sympathetic ganglia

TREATMENT of neonatal animals with nerve growth factor (NGF) promotes general growth and differentiation of the peripheral sympathetic nervous system. NGF markedly increases the outgrowth of axons and at the same time causes the appearance of many microtubules in electron-microscopic pictures¹. Moreover, as a manifestation of enhanced differentiation NGF produces a selective induction of tyrosine hydroxylase (TH) and dopamine β -hydroxylase in sympathetic ganglia². These two enzymes catalyse key steps in the biosynthesis of noradrenaline and are located selectively in adrenergic neurones³. In contrast, the third enzyme involved in the synthesis of noradrenaline, dopa decarboxylase (DDC), has a more general distribution and is not specifically regulated by NGF².

It is not clear whether the promotion of axon outgrowth and the accompanying increased formation of neurotubules results from specific induction of subunit synthesis⁹ or from regulation of the assembly of subunits. The purpose of this study was to resolve this question by examining simultaneously the effect of NGF on tubulin subunit content and TH activity.

NGF was prepared as the 2.5S subunit⁴ from mouse submaxillary gland. A single subcutaneous injection (10 mg per kg body weight) was administered to 5-d-old rats weighing 20–25 g. At appropriate times the animals were killed by a blow on the head and the superior cervical ganglia were removed under a binocular dissecting microscope. For determination of enzyme activity and protein content one pair of ganglia were homogenised in 0.5 ml 0.005 M Tris buffer containing 0.1% Triton in a power-driven glass homogeniser. The homogenates were centrifuged for 20 min at 10,000g at 4° C. Proteins⁵, TH⁶ and DDC⁷ were assayed; both TH and DDC assays were modified as described by Oesch et al.⁸. To determine tubulin subunit content 4–7 pairs of ganglia were homogenised in 0.5 ml of 0.05 M Na-pyrophosphate, 0.2 M NaCl, 1 M sucrose, pH 7 and supernatants were prepared as above. The presence of 1 M sucrose does not affect the absolute value of the colchicine binding activity in the extracts, but does increase the half life of that activity to approximately 220 h (ref. 9).

Figure 1a shows the time course of the changes in TH activity and protein content per pair of ganglia after a single injection of 10 mg per kg of NGF. A statistically significant ($P < 0.05$) increase in TH activity compared with untreated controls was detectable as early as 12 h after injection. The

maximum increase (350% of control) was reached at 48 h. Thereafter, the activity decayed gradually to 130% at 120 h. In contrast, a statistically significant ($P < 0.05$) increase of the protein content was not reached before 48 h and amounted to only 20%. The induction of TH was completely abolished if the animals were injected subcutaneously with 1.6 mg per kg of actinomycin D, 3 h before administration of NGF, and were killed 24 h later.

In contrast to the activity of TH, that of DDC changed virtually in parallel with the protein levels. The same is true for tubulin which did not change its specific activity over the whole period of 120 h (Fig. 1b).

In a further series of experiments the animals were injected daily with 10 mg per kg of NGF given subcutaneously for 5 consecutive days and were killed 24 h after the last dose. Under these experimental conditions the total activity of TH increased to 750% of controls, the specific activity to 375%. The corresponding values for tubulin were 275 and 140% respectively (Fig. 2).

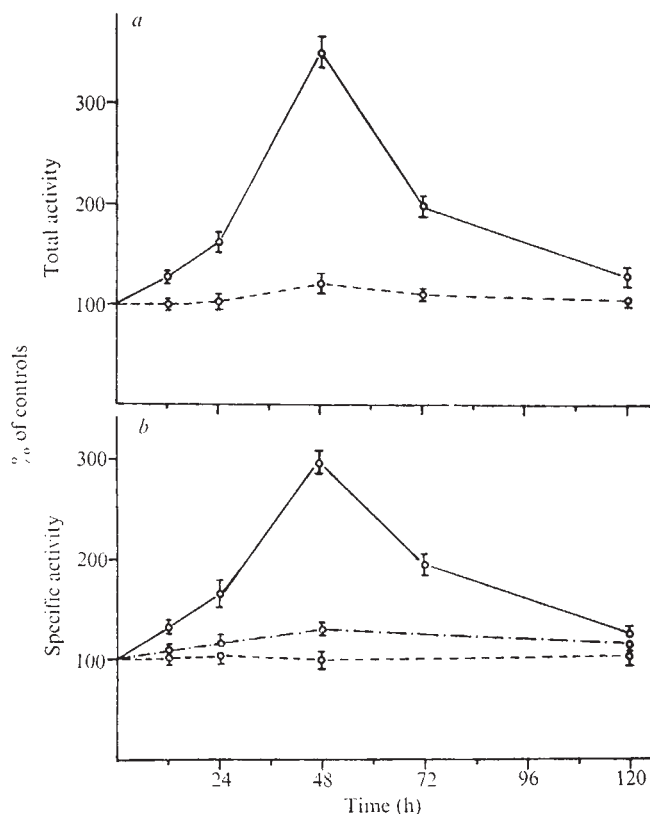


Fig. 1 Effect of a single dose of NGF on the levels of a, —, tyrosine hydroxylase (TH), ---, protein; b, —, tyrosine hydroxylase, ---, dopa decarboxylase (DDC),, tubulin in the rat superior cervical ganglia. The animals were injected subcutaneously with 10 mg kg⁻¹ of NGF 5 d after birth. TH activity and protein were determined in the supernatant after homogenising the ganglia in 0.005 M Tris-0.1% Triton X-100 and centrifuging the homogenate for 20 min at 10,000g. For the determination of TH activity the substrate (L-tyrosine) concentration amounted to 15 μ M, that of the cofactor (6, 7-dimethyl-5, 6, 7, 8-tetrahydropteridine) to 720 μ M. For the determination of DDC the substrate (L-³H-dopa) concentration was 1 mM, that of the cofactor (pyridoxal-5'-phosphate) was 0.24 mM and that of translycypromine was 1.2 mM. At the time of injection activity of TH was 0.3040 ± 0.019 nM dopa formed per h per pair of ganglia; that of DDC was 86.34 ± 6.04 nM dopamine formed per h per pair of ganglia. Tubulin was determined after incubation of the 10,000g supernatant with 8.3×10^{-6} M colchicine (2 Ci mmol⁻¹) for 90 min at 37° C by the filter assay of Borisov¹⁰. The specific activity of the tubulin in treated ganglia 48 h after injection was 3.6×10^{-5} d.p.m. bound per mg of protein. The values given represent the mean $\pm$ s.e.m. of six or seven animals per group.

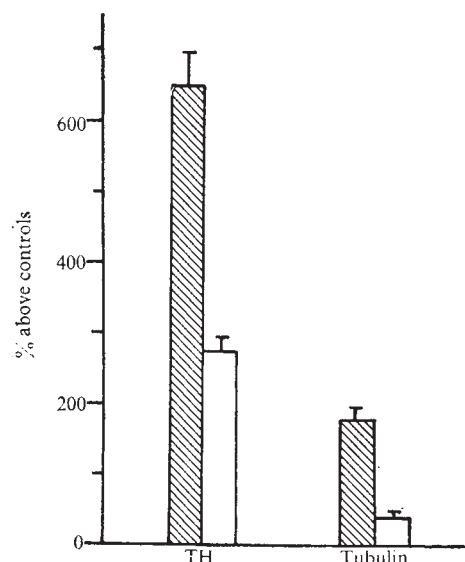


Fig. 2 Effect of repeated administration of NGF on level of TH and tubulin in the superior cervical ganglion. NGF was given subcutaneously on 5 consecutive days starting on the fifth day after birth. The ganglia were dissected 24 h after the last injection and treated as described in text. In the control animals the TH activity was 0.233 ± 0.007 nM dopa formed per h per pair of ganglia, the protein content was 14.8 ± 5.9 μ g per pair of ganglia, and the tubulin content was 5.52×10^{-4} d.p.m. bound colchicine per pair of ganglia. The values given represent the means $\pm$ s.e.m. of six or seven animals per group. Shaded column, total activity; unshaded column, specific activity.

This study has shown that the effect of NGF on TH synthesis is quantitatively distinct after both single and multiple injections from the effect on tubulin synthesis. The dissociation of the appearance of increased numbers of neurotubules in electron microscopic pictures¹⁴ from any specific increase in the subunit content of the cells is consistent with the interpretation that NGF influences the assembly of preformed neurotubular subunits rather than enhancing their synthesis. This agrees with findings in other systems that outgrowth of cellular processes accompanied by the formation of microtubules does not require protein synthesis¹¹⁻¹³. Moreover, the induction of process formation in neuroblastoma cells by a glial-conditioned medium¹⁵ does not increase the tubulin content of the cells (F. S. and D. Monard, manuscript in preparation).

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Reduced axoplasmic transport of choline acetyltransferase activity in dystrophic mice

MUSCULAR dystrophy has been regarded as a "primary degenerative myopathy"¹. But there is evidence from studies of both human muscular dystrophy and animal models of muscular dystrophy that the muscle disease may have a neural basis². The mechanism of the suspected neural disorder remains to be elucidated. Since there is independent evidence that in normal animals the trophic effect of nerve on muscle depends on intact axoplasmic transport³, we have initiated a study of axoplasmic transport in dystrophic mice. We have found a disturbance of axoplasmic transport of choline acetyltransferase activity in dystrophic mice.

Choline acetyltransferase (CAT) is the enzyme that catalyses the synthesis of the neurotransmitter acetylcholine⁴. In neural tissue, CAT is probably confined to cholinergic neurones⁵. It is likely that CAT, like other neural proteins, is synthesised in the nerve cell body and transported peripherally in the axoplasm to the nerve terminal^{6,7}. If a peripheral nerve is crushed by a ligature, CAT activity slowly increases on the proximal side of the constriction⁸. From the rate of accumulation of CAT activity, one can estimate the average velocity of proximo-distal axoplasmic transport of CAT⁹⁻¹¹. Here we present a study that shows a significant reduction in the average velocity of axoplasmic transport of CAT activity and a significant increase in the CAT activity in sciatic nerves of mice afflicted with an autosomal recessive form of muscular dystrophy¹². These findings may reflect a fundamental abnormality of axoplasmic transport of cholinergic neurones in dystrophic mice.

Male 129/ReJ dystrophic mice (dys/dys) with normal littermates (+/?), and normal retired breeders of known genotypes, (+/+) and (+/dys), were obtained from Jackson Memorial Laboratory, Bar Harbor, Maine. A ligature was placed on the right sciatic nerve at mid-thigh level¹³. Animals were killed at various times following ligation and both sciatic nerves excised. One 1 cm length was cut from the middle of the non-ligated sciatic nerve. The 9 mm length of nerve immediately proximal to the ligature was divided into three 3 mm segments. The heat-labile CAT activity of the 8000g $\times$ 10 min supernatant fraction of homogenised nerve segments was assayed with a simultaneous determination of the recovery of added purified mouse brain CAT activity. One unit of enzyme activity represents the production of 1 pmol of acetylcholine (ACh) from choline and ¹⁴C-acetyl coenzyme A per 3 mm length of nerve per min. We defined the accumulation of CAT activity as the increase in CAT activity within the 3-mm segment immediately proximal to the ligature. The initial CAT activity of the ligated nerve was estimated from the CAT activity of the non-ligated sciatic nerve. Values are presented as the mean $\pm$ s.e.m. The statistical significance of values was determined by the Student *t* test.

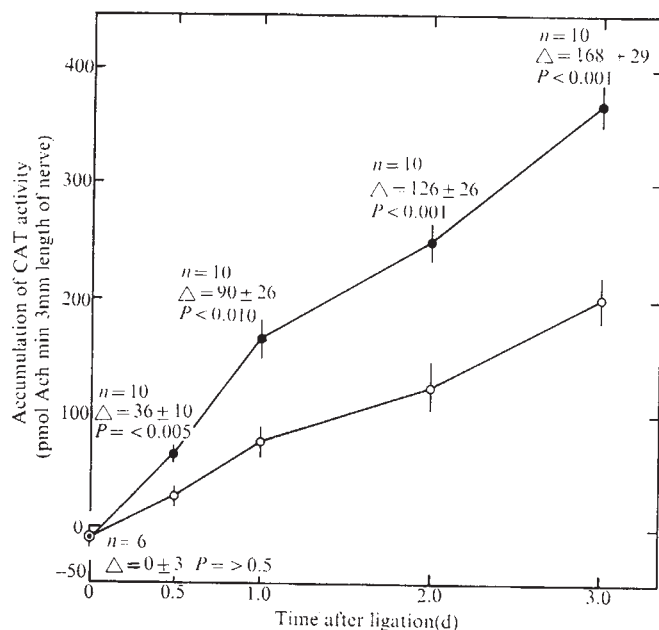


Fig. 1 Accumulation of CAT activity in sciatic nerves of 6 to 7-week-old dystrophic mice (○) and normal littermates (●) at various times after unilateral sciatic nerve ligations. The number of littermate pairs (n), and the statistical significance (P) of the difference (Δ = mean $\pm$ s.e.m.) between the accumulation of CAT activity of the littermate pairs are indicated at each time of killing. Nerve segments were homogenised (1 mm per 100 μ l) in all glass Duall tissue grinders with 0.2 M Tris-HCl (pH 8.5), 0.5 M KCl, 1 mM EDTA, 1 mg ml⁻¹ bovine serum albumin (BSA) and 1% butanol-1 (v/v); the homogenates were centrifuged at 8,000g for 10 min. CAT activity was assayed in a 20 μ l reaction mixture with a final concentration of 100 μ M ¹⁴C-acetyl-coenzyme A (specific activity 53 mCi mmol⁻¹, New England Nuclear, Boston, Mass.), 15 mM choline iodide, 0.1 M Tris-HCl (pH 8.5), 0.5 M KCl, 1 mM EDTA, 0.1 mM eserine sulphate, 0.5 mg ml⁻¹ BSA, 0.5% butanol-1 (v/v) and the 8,000g supernatant fraction of 0.083 mm of nerve. The CAT activity reaction mixture was incubated 6 min at 38°C, the reaction was stopped with 10 μ l of 2 N formic acid, and the product was extracted into octanone and measured as described by Glover and Green²⁵. Occasionally in parallel experiments, the CAT reaction product from non-ligated and ligated nerve samples was further characterised by high voltage electrophoresis²⁶. There was quantitative agreement between the radioactivity that migrated with the R_f of acetylcholine and the radioactivity extracted into octanone. In the case of both dystrophic and normal mice (1) omission from the homogenisation solution of the KCl resulted in the failure to retain 80% of the homogenate CAT activity in the 8,000g supernatant fraction; (2) incubation at pH 7.0 instead of 8.5 resulted in 30% less CAT activity, and (3) omission from both the homogenisation and incubation mixture of the EDTA, BSA, or butanol-1 resulted in 80%, 35%, and 10% less CAT activity, respectively.

In both the dystrophic mice and their normal littermates, CAT activity accumulated as a linear function of time for at least 3 d after unilateral ligation of sciatic nerves (Fig. 1). But the accumulation of CAT activity in dystrophic mice was only $48 \pm 5\%$ of that in normal littermates whose nerves had been ligated for the same period of time ($n = 40$, $P < 0.001$). The relative accumulation of CAT activity was computed from (1) the accumulation of CAT activity in the ligated sciatic nerve of the dystrophic mouse as a percentage of (2) the accumulation

in the normal littermate, for the 40 pairs after time zero in Fig. 1.

The accumulation of CAT activity also may be described by equations fitted by the method of least squares to the data in Fig. 1: $y_c = 122t_c + 10$ ($n = 46$, $r = 0.93$, $P < 0.001$) and $y_d = 68t_d - 6$ ($n = 46$, $r = 0.77$, $P < 0.001$) for control and dystrophic mice, respectively, where y represents units of CAT activity, and t represents time in days. The average velocity of transport of CAT activity was computed from the time it took to increase the CAT activity in the ligated nerve by an amount equal to the initial CAT activity. Solution of the equations for $y_c = 202$ units and $y_d = 231$ units (see below) yielded $t_c = 1.6$ d and $t_d = 3.5$ d. Thus the average velocity of proximo-distal transport was 3 mm per 1.6 d = 1.9 mm d⁻¹ for the controls and 3 mm per 3.5 d = 0.9 mm d⁻¹ for the dystrophic mice.

The difference between the rates of accumulation of CAT activity in the sciatic nerves of dystrophic mice and normal littermates was not due to a difference in the spatial distribution of accumulated CAT activity. For at least 3 d after ligation, the accumulation of CAT activity was confined to the 3mm segment proximal to the ligature in both dystrophic and normal mice (Fig. 2).

The CAT activity per unit length of non-ligated sciatic nerves from dystrophic mice was 14% more than that of the controls. The non-ligated sciatic nerves of the dystrophic mice and normal littermates in Fig. 1 yield 231 ± 5 and 202 ± 6 units CAT activity, respectively ($\Delta = 29 \pm 5$ units, $n = 46$, $P < 0.001$). Though we do not know what fraction of the soluble proteins of nerves is axonal in origin, we found an increase in the soluble proteins in nerves of dystrophic mice that paralleled the increase in CAT activity. In companion experiments, the right sciatic nerves of 6 to 7-week-old dystrophic mice and normal littermates were assayed for CAT activity; the left nerves were weighed, and the 8,000g supernatant fractions were assayed for protein¹⁴. The weights of non-ligated sciatic nerves from dystrophic mice were not significantly different from those of the normals (1.1 ± 0.1 compared with 1.2 ± 0.1 mg cm⁻¹, $\Delta = 0.1 \pm 0.1$, $n = 8$, $P > 0.5$). Because Tris-HCl buffer and BSA interfered with protein determinations, 0.2 M KPO₄ buffer (pH 8.0) was substituted for Tris-HCl buffer in all homogenisation solutions, and the BSA was omitted from the homogenisation solutions used for measurement of protein content. The CAT activity was 19% greater in the nerves of the dystrophic mice (250 ± 9 compared with 210 ± 15 units, $\Delta = 40 \pm 13$, $n = 8$, $P < 0.02$), and the soluble protein was 18% greater in the nerves of the dystrophic mice (53 ± 2 compared with 45 ± 3 μ g cm⁻¹, $\Delta = 8 \pm 2$, $n = 8$, $P < 0.01$).

The differences in initial CAT activity and in the accumulation of CAT activity were not due to differences in the content of endogenous activators or inhibitors of CAT activity. In every case, the recovery of purified mouse brain CAT activity added to the 8,000g supernatant fraction of corresponding nerve segments was the same for dystrophic mice and their controls. Table 1 shows the recoveries of added CAT activity from samples of nerve segments 3 d after unilateral sciatic nerve ligation.

Table 2 presents the results of several experiments with different ages and genotypes of 129/ReJ mice. In the sciatic nerves of 9 to 12-month-old normal retired breeder 129/ReJ mice, neither CAT activity nor accumulation of CAT activity differed between the genotypes (+/dys) and (+/+). Therefore, normal mice (+/?) probably constitute a reasonable control population for the study of dystrophic mice (dys/dys). As controls for this study, we chose normal mice which were also matched for

Table 1 Recovery of added purified CAT activity from 8,000g supernatant fraction of sciatic nerve segments

Group*	Non-ligated nerve		Ligated nerve (mm proximal to ligature)†	
			3	0
Dystrophic mice	120 $\pm$ 8%‡	107 $\pm$ 6%	115 $\pm$ 10%	98 $\pm$ 15%
Normal littermates	117 $\pm$ 11%	112 $\pm$ 15%	106 $\pm$ 10%	103 $\pm$ 11%

* The ten pairs of dystrophic mice and normal littermates killed 3 days after unilateral ligation of the sciatic nerve included in Fig. 1.

† The number refers to the distance between the ligature and the 3-mm segments of nerves (see Fig. 2).

‡ The recovery of added CAT activity was calculated as a percentage of the purified CAT activity added. CAT activity was purified 99-fold (final specific activity 0.13 μ mol Ach min⁻¹ mg⁻¹) from brains of 6 to 7-week-old C57 BL/10J mice from Jackson Memorial Laboratory, Bar Harbor, Maine²⁷.

Table 2 Initial CAT activity and accumulation of CAT activity 1 d after unilateral ligation of sciatic nerves in various groups of 129/ReJ mice

Group	Phenotype	Genotype	Weight (g)	Sciatic nerve CAT activity (pmol Ach per min per 3-mm length of nerve)	
				Initial	Accumulation
Eight 3 to 4-week-old littermate pairs	normal	+/?	9.7 ± 0.7	168 ± 10	204 ± 19
	dystrophic	dys/dys	7.1 ± 0.7*	214 ± 19 ⁺	111 ± 31 ⁺
Ten 6 to 7-week-old littermate pairs	normal	+/?	20.2 ± 0.6	185 ± 12	164 ± 20
	dystrophic	dys/dys	10.2 ± 0.5 [‡]	214 ± 8*	74 ± 12 ⁺
Eight age-matched (9 to 12-month-old) pairs of retired breeders	normal	+/?	25.1 ± 1.1	498 ± 20	149 ± 30
	normal	+/?	30.0 ± 1.2	500 ± 26§	157 ± 18§

Significantly different from their normal littermates with * $P < 0.02$, + $P < 0.01$, or $^{\ddagger}P < 0.001$.

§ Not significantly different ($P > 0.5$) from the age-matched controls.

age (littermates). Table 2 shows that the increase in CAT activity and the reduction in the accumulation of CAT activity were evident in 6 to 7-week-old dystrophic mice whether one uses normal controls matched for age (littermates) or normal controls matched for body weight (3 to 4-week-old normal mice).

The neuronal mechanisms which are responsible for the increase in CAT activity and the reduced rate of accumulation of CAT activity in sciatic nerves of dystrophic mice remain to be elucidated. The reduced accumulation of CAT activity probably reflects a decrease in the amount of CAT activity that moves down the nerve axon towards the nerve terminal each day. This interpretation is compatible with the recent autoradiographic evidence that there is a reduction in the amount of protein slowly transported down nerves of dystrophic mice¹⁵.

The reduced rate of accumulation of CAT activity in nerves of dystrophic mice could be explained by (1) a decrease in the number of cholinergic fibres in the nerves¹⁶⁻¹⁸; (2) a decrease in the amount of CAT available for transport from nerve cell bodies; (3) a decrease in the velocity or in the fraction of CAT transported down nerve axons. But of these alternatives, only the latter one, by itself, can also explain the increase in initial CAT activity we observed in nerves of dystrophic mice.

Other abnormalities of peripheral nerves in dystrophic mice have been reported. Nerves of dystrophic mice have areas of abnormal myelination¹⁹, there are differences between the gel electrophoresis patterns of proteins from nerves of dystrophic and control mice²⁰, and the nerves from dystrophic mice are less able than nerves from controls to support muscle regeneration^{2,21}. Our finding of reduced axoplasmic transport of CAT activity in dystrophic mice may be indicative of a primary neuronal abnormality. It is possible, however, that our results reflect a disturbance of neuronal function that is secondary to muscle disease.

With regard to the latter alternative, our observation of early changes in CAT activity and its transport suggests that these changes are at least not late consequences of the muscle disease (Table 2). Also, the loss of muscle cells that accompanies murine dystrophy seems an unlikely cause of the increased CAT in nerves of dystrophic mice because there is evidence that the absence of contact of muscle cells with neurones results in lower CAT activity of the neurones^{22,23}. It is also unlikely that the loss of muscle cells is the cause of reduced axoplasmic transport of CAT activity since we have recently shown that section of the sciatic nerve in normal mice does not reduce the rate of axoplasmic transport of CAT activity proximal to the sectioned end (unpublished results).

The CAT activity of dystrophic muscle has been shown to be reduced compared with normal muscle²⁴. The reduction in dystrophic muscle CAT activity may be related to the reduction in axoplasmic transport of CAT activity documented here. Whether reduced axoplasmic transport of CAT activity is actually involved in the pathogenesis of the muscle abnormalities in murine muscular dystrophy remains to be determined. The neural abnormalities in dystrophic mice may be independent of the muscle disease. But as previously mentioned, there is evidence that the trophic effect of nerve on muscle depends on intact axoplasmic transport³. Therefore, it is possible that the muscle disease in dystrophic mice may be related to a disturbance of the axoplasmic transport of motor (cholinergic) neurones.

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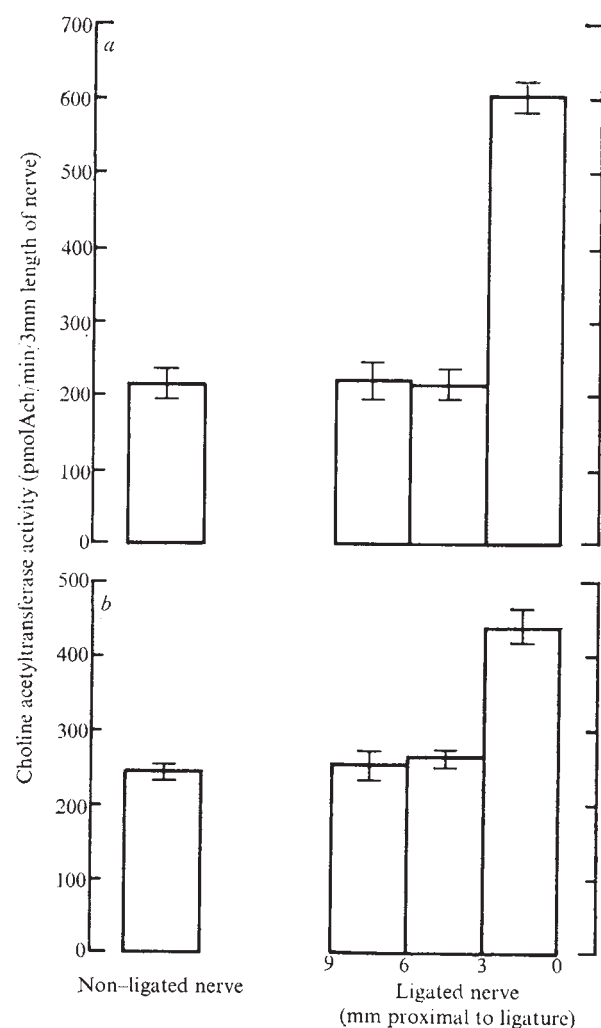


Fig. 2 Effect of unilateral ligation on sciatic nerve CAT activity in *a*, normal and *b*, dystrophic mice. Each value is the mean ± s.e.m. CAT activity of nerve segments from the same ten pairs of dystrophic mice and normal littermates killed 3 d after unilateral ligation of the sciatic nerves included in Fig. 1.

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Haemocyanin synthesis and the branchial gland of *Octopus*

MUCH is known about the biochemistry of haemocyanin¹ but little about how or where it is made. It has recently been suggested² on morphological grounds that in cephalopod molluscs it is synthesised in the paired branchial glands. Here we present the first experimental evidence to support this hypothesis and throw doubt on earlier theories that these organs are endocrine³⁻¹⁰.

The gland cannot be an analogue of the adrenal medulla or cortex^{8,9}. It seems to be without nerves^{2,11} and pharmacological and fluorescence tests for monoamines are negative; it contains no lipids¹² or steroids¹³ and the well-developed endoplasmic reticulum (ER) is not smooth². Although transient cardiostimulation can be produced by crude extracts, this effect is not dose-dependent or specific to the gland, is much less than that of 5-HT and is not sustained as are the responses to extracts of the vena cava¹⁴. Furthermore, transplants are not effective in preventing death after bilateral extirpation of the glands.

The most remarkable features of the *Octopus* branchial gland are the extraordinary development of rough-surface ER and the presence of vacuoles within the cell containing granules that look like haemocyanin². These findings have been independently confirmed and extended to a squid¹⁵. Martin and Muzii (unpublished observations) have also

observed large intracellular inclusions of what appear to be macromolecules in crystalline array. These were of two sizes, one of which agreed with Fernandez-Moran's estimate¹⁶ for the cephalopod haemocyanin molecule.

It has also been shown¹⁷ that large amounts of labelled leucine injected into the blood disappear rapidly and are taken up by the branchial glands. If the glands are haematopoietic they should take up amino acids and the rate of uptake should be severely reduced after the elimination of the glands. We set out to establish this in our first experiments.

To impair the function of the glands, we applied slivers of dry ice directly to their surface and froze them. Subsequent examination under the light microscope and EM revealed extensive necrosis and we estimated that less than 10% of the total gland mass remained functional after freezing. This method was adopted because previous experiments had shown that complete, simultaneous, bilateral excision is generally fatal.

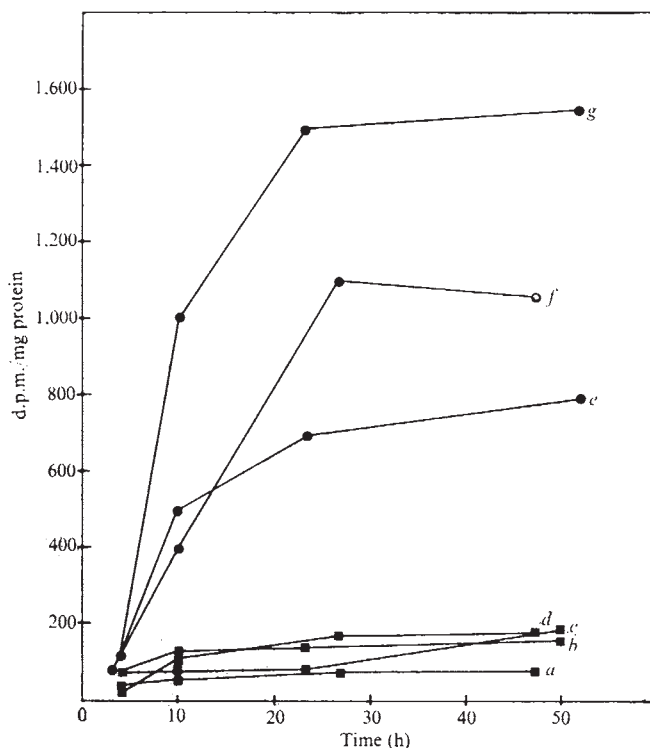


Fig. 1 *In vivo* L-¹⁴C-leucine incorporation into the haemolymph proteins of *Octopus vulgaris*. Experimental animals, ■, (a, ♂ 230 g; b, ♀ 165 g; c, ♂ 160 g; d, ♂ 620 g) had their branchial glands frozen. Control animals, ●, (e, ♀ 155 g; f, ♀ 180 g; g, ♂ 170 g) were anaesthetised only. Counts were corrected for background.

Healthy *Octopus vulgaris* were anaesthetised with 3% urethane, the mantle septum was cut and the mantle reversed to expose the visceral mass. Control operations ($n=3$) stopped at this point but the experimental group ($n=4$) had dry ice applied to the glands. The mantle was turned back and the animals allowed to recover in a stream of seawater.

Twenty-four hours afterwards, each animal was again anaesthetised and a single dose of 5.0 μ Ci of L-¹⁴C-leucine in 0.4 ml 1 M Tris-HCl buffer (pH 7.5) was injected into the lumen of the branchial heart. Haemolymph samples of 200 μ l were withdrawn under anaesthesia from the contra-lateral branchial heart at varying intervals over a 54 h period; blood cells were allowed to sediment overnight and the protein content of the supernatant determined by the

Biuret method¹⁸ using BSA as a standard.

Radioactivity was measured in aliquots of whole haemolymph and compared with the radioactivity in TCA-precipitated haemolymph proteins. Samples of whole haemolymph (100 μ l) were dissolved in 10 ml of InstaGel scintillation cocktail (Packard, Chicago) and TCA-precipitated protein was collected on glass fibre disks¹⁹ and counted in a toluene-based scintillation fluid containing 5 g l⁻¹ PPO and 0.2 g l⁻¹ POPOP. Results from both methods were in excellent agreement.

Figure 1 illustrates the effect of freezing the glands on the uptake of labelled leucine. Experimental animals showed almost no increase in activity throughout the entire period of the experiment yet, in spite of considerable individual variation (not obviously related to size or sex), animals of the control group all exhibited a high uptake of radioactivity into the blood protein.

Although haemocyanin represents the major protein constituent of *Octopus* haemolymph, to confirm that the radioactivity reflected the synthesis of haemocyanin rather than some other protein, we resorted to an immunological characterisation, using the Ouchterlony double-diffusion method on an agar plate²⁰ followed by autoradiography of the dried plate. Haemocyanin antibodies were obtained by intravenously inoculating rabbits with 10 mg pure octopus haemocyanin three times at 10 d intervals; serum was collected 1 week after the last inoculation. In preliminary experiments anti-haemocyanin serum was tested against purified haemocyanin, whole haemolymph and concentrated haemocyanin-free haemolymph. A single precipitation line showing a complete arc of identity developed between serum and haemocyanin and whole haemolymph. No precipitation line was formed with haemocyanin-free haemolymph even when it was concentrated eight-fold.

In Fig. 2a is shown a double diffusion plate stained with Amidoschwarz in which haemolymph from control and operated animals (I and IV) gave a strong positive reaction, each yielding a single precipitation line that was identical to that of purified haemocyanin (II and III).

Autoradiography of the plate, by exposing the Kodak recording film to the dry gel plate for 2 weeks, revealed that the radioactivity was localised in a single line corresponding to the control animals' haemocyanin-antibody

complex (Fig. 2b). There can be no doubt, therefore, that the amino acid is being incorporated into haemocyanin and that only in octopuses with intact branchial glands does labelled leucine get into the haemocyanin.

From this we conclude that the branchial gland of *Octopus* is undoubtedly involved in the biosynthesis of the respiratory pigment haemocyanin. Little is known about this process and it is hoped that the large size and accessibility of the branchial gland may make this gland invaluable for studying it.

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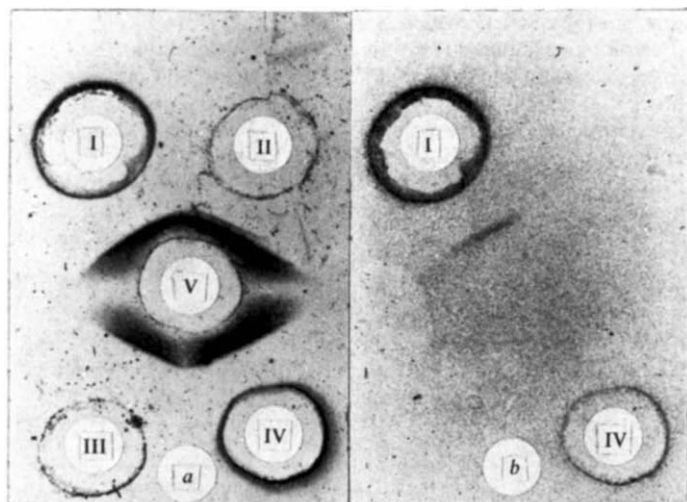


Fig. 2 Incorporation of L-¹⁴C-leucine into *Octopus* haemocyanin: immunoautoradiography. *a*, Amidoschwarz-stained immunodiffusion. Pure haemocyanin in wells II and III; radioactive haemolymph from control animal in well I; radioactive haemolymph from experimental animal in well IV; and anti-haemocyanin serum in well V. *b*, Autoradiography of *a*. Note the dark line indicating the radioactivity in the precipitation arc between wells I and V.

Secretion-dependent uptake of extracellular fluid by the rat neurohypophysis

THERE is considerable evidence that hormone secretion from the neurohypophysis occurs by exocytosis of neurosecretory granules present in the nerve terminals^{1–4}. Exocytosis involves fusion of the granule membrane with the plasma membrane so that the entire soluble content of the granule is discharged into the extracellular space. Persistent hormone secretion does not lead to irreversible expansion of the plasmalemma of neurosecretory terminals, so the granule membrane must somehow be

retrieved after fusion. There is morphological evidence based on the use of electron dense markers in various preparations, that following exocytosis membrane is returned to the cell interior where it is present as microvesicles and larger cisternae⁵⁻¹⁰.

As large marker substances are taken into the nerve terminals, it seems likely that there ought also to be uptake of smaller molecules in proportion to their concentration in the external medium. If this is the case, the uptake of radioactive tracers might provide quantitative data on the magnitude of endocytosis occurring at actively secreting neuroendocrine terminals.

Neurohypophyses obtained from adult rats following decapitation were incubated in groups of four in 1 ml of oxygenated Locke solution¹¹ maintained at 37° C in a water bath. After 20 min, the solution was drawn off and immediately replaced by one containing: choline chloride, 150 mM; KHCO₃, 6 mM; CaCl₂, 2.2 mM; MgCl₂, 1 mM; glucose, 10 mM. ¹⁴C-Inulin (7.5 µCi ml⁻¹), ¹⁴C-mannitol (2.4 µCi ml⁻¹) or ¹²⁵I-albumin (2.4 µCi ml⁻¹, all three from Amersham) were present in the bathing fluid for the next 40 min. During the last 10 min of this second incubation, hormone release was evoked by raising the external KCl concentration to 56 mM, the choline chloride concentration being lowered to maintain constant osmolarity. This incubation medium was then withdrawn and its oxytocin content determined in a rat bioassay¹². After stimulation, the preparations were washed for 150 min in Locke solution to remove the tracer molecules present in the extracellular spaces, the solution being renewed every 10 min. The neurohypophyses were then weighed, homogenised in 1 ml of HClO₄ 11% (w/v), centrifuged, and the radioactivity of the supernatant determined.

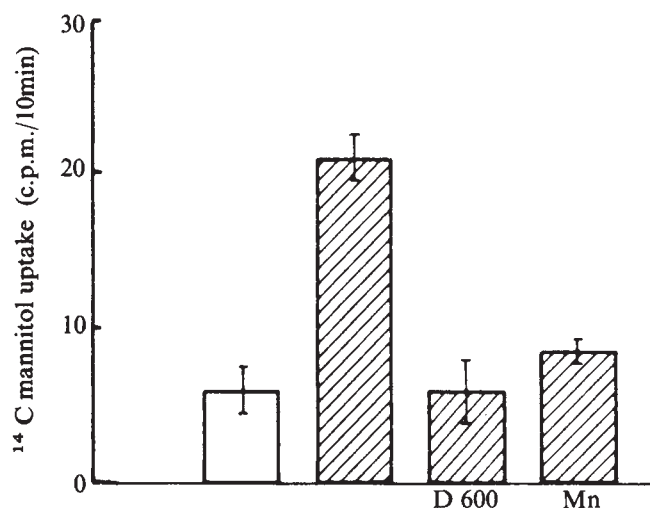


Fig. 1 Effects of calcium antagonists on mannitol uptake. Same experimental design as data of Table 1. □, Control uptake ($n = 4$); striped columns, stimulated preparations (56 mM KCl). Note absence of extra uptake of mannitol in preparations depolarised in presence of D600 (0.1 mM, third column $n = 6$) or Mn²⁺ (5 mM, fourth column, $n = 4$). Each column represents mean $\pm$ s.e.m.

Exposure of neurohypophyses to the depolarising solution led consistently to an increased cellular uptake of extracellular marker substance. The results, summarised in Table 1, indicate that uptake is increased 3.0–5.4 fold in the stimulated preparations. The extra uptake of mannitol was greater than that of inulin or albumin but more experiments are needed to decide whether this difference is significant. The extra uptake could be caused either directly by a depolarisation-induced increase in the permeability to the tracer or indirectly as a result of secretion. The second hypothesis seems most likely because the extra uptake is abolished by including in the external medium either 5 mM manganous chloride or 0.1 mM of the organic calcium antagonist D600, (Fig. 1), both of which abolish

Table 1 Amount of radioactivity taken up by stimulated and control neurohypophyses

	Trapped radioactivity (c.p.m. per mg tissue)		Equivalent volume of extracellular fluid taken up during stimulation ($\mu\text{m}^3 \text{mg}^{-1}$)
	Stimulated preparations	Controls	
¹⁴ C-Mannitol	20.4 ± 1.5 (8)	5.7 ± 0.4 (4)	7.0×10^6
¹⁴ C-Inulin	3.3 ± 0.6 (6)	1.1 ± 0.1 (5)	1.1×10^6
¹²⁵ I-Albumin	4.1 ± 0.2 (8)	0.75 ± 0.1 (4)	1.1×10^6

Preparations were kept 40 min in a Na-free Locke solution containing radioactive mannitol (0.015 mg ml⁻¹), inulin (~40 mg ml⁻¹) or albumin (~2 mg ml⁻¹). Stimulated neurohypophyses were incubated for the last 10 min in a high-K Na-free solution in presence of one of the extracellular marker substances. The Table shows the calculated uptake in control and stimulated preparations during the last 10 min of the incubation with radioactive marker. The difference in uptake between stimulated and control preparations is highly significant in all three cases (t test, $P < 0.001$). Each value represents mean $\pm$ s.e.m. with the number of experiments in brackets.

calcium uptake induced by depolarisation of nerves¹³ and calcium-dependent hormone release from the neurohypophysis¹⁴. The view that uptake of extracellular tracer induced by depolarisation is secondary to secretion is supported by two other observations: first, both hormone release¹⁵ and tracer uptake are greater when secretion is evoked in a Na-free medium than when Na is present and, second, in Na-free media uptake of tracer induced by depolarisation increases linearly with tracer concentration in the external medium (Table 2).

These data are fully consistent with the hypothesis that, following exocytosis, membrane is retrieved by a process functionally similar to pinocytosis. On the assumption that uptake of tracer occurs without change in its concentration, it is possible to express the uptake in terms of a volume of extracellular fluid taken up and relate this to an equivalent volume of granules that must have undergone exocytosis. The data of Tables 1 and 2 give, for the three extracellular markers used, an average uptake of extracellular fluid equivalent to $3.1 \times 10^6 \mu\text{m}^3$ per mg tissue. In the same conditions, the amount of vasopressin and oxytocin released amounted to 90 milliunits, that is approximately 10% of the total neurohypophysial hormone content of the glands believed to be contained in 2×10^{10} neurosecretory granules¹⁶. Assuming that release occurs solely by exocytosis, and taking 85 nm to be the mean radius of the core of the granules, the induced release should lead to a loss of $5.2 \times 10^6 \mu\text{m}^3$ per mg of tissue which is in surprisingly close agreement to the volume calculated from the uptake of extracellular markers.

If it is assumed, first, that both exocytosis and endocytosis involve spherical vesicles and, second, that membrane area is conserved, the relative volumes of fluid lost during exocytosis and gained during subsequent endocytosis should be in direct proportion to the ratio of the diameters of the vesicles involved in the two processes. Our data are consistent with membrane retrieval occurring through vesicles of diameter comparable to the granules undergoing exocytosis; but the data are not yet good enough to rule out other possibilities.

Morphological evidence supporting our conclusions was obtained by using the extracellular tracer horseradish peroxidase (HRP; Sigma, type II). HRP (2 mg ml⁻¹) had no effect on either resting levels of hormone secretion or secretion induced by depolarisation. In control experiments, following exposure to

Table 2 Dependence on the extracellular mannitol concentration of the secretion-dependent uptake of ¹⁴C-mannitol.

Mannitol concentration (mM)	Extra uptake in stimulated preparations (fmol mg ⁻¹)	Equivalent volume of extracellular fluid taken up ($\mu\text{m}^3 \text{mg}^{-1}$)
0.07	$4.9 \times 10^2 \pm 0.2$ (8)	7.0×10^6
1	$67.7 \times 10^2 \pm 9.7$ (3)	6.8×10^6
3	$127.1 \times 10^2 \pm 15.2$ (3)	4.2×10^6
5	$317.4 \times 10^2 \pm 11.7$ (3)	6.3×10^6

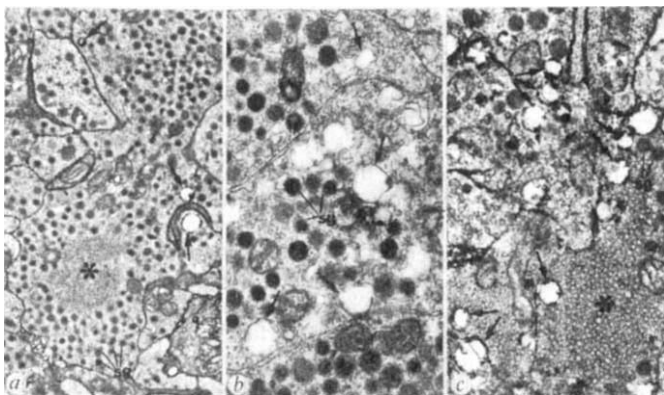


Fig. 2 Electron micrographs of neurohypophyses incubated in the presence, (a, c), or absence, (b), of horseradish peroxidase (HRP). Unfrozen sections (40 μ m thick) of glutaraldehyde-fixed specimens were treated to demonstrate sites of peroxidase activity¹⁷. a, Low magnification ($\times 7,500$) view of non-stimulated neurosecretory endings. Black HRP reaction product is present in the intercellular spaces, and in a few vacuoles within the cytoplasm (arrows). b, Stimulated neurohypophysis incubated in the absence of the tracer, to reveal endogenous peroxidase contained no HRP-tagged structures. The arrows indicate several membrane-bound vacuoles ($\times 16,000$). c, Stimulated neurohypophysis. The arrows point to numerous peroxidase-labelled vacuoles. The size and location of the vacuoles are comparable to those shown in B ($\times 13,250$). * collections of microvesicles; sg, neurosecretory granules.

HRP for 40 min, the reaction product was mainly present in the extracellular space and very little was detectable in vesicles within the cytoplasm (Fig. 2a). In preparations that had been depolarised for 10 min (Fig. 2b and c) there was a variable degree of degranulation of neurosecretory endings and HRP reaction product was found in numerous membrane-bound vacuoles of a size (~ 100 nm radius) somewhat larger than that of the neurosecretory granules (~ 85 nm radius) seen within the endings. The vacuoles were preferentially localised near the plasmalemma. As previously reported⁵, microvesicles labelled with peroxidase, both coated and uncoated, could also be found but even after brief depolarisations their number was always low in proportion to the labelled vacuoles. As we found relatively few HRP-positive microvesicles, our results contrast with those of Nagasawa *et al.*⁵. We have no ready explanation for this discrepancy, but note that our experiments were performed in solutions free from sodium, leading thereby to a more powerful release reaction¹⁵. It may well be that two routes of endocytosis exist, and their relative importance may depend upon the intensity of secretion¹⁸.

Previous workers have described an extra uptake of calcium associated with depolarisation of the posterior pituitary^{14,19} and in view of our evidence for uptake of extracellular fluid it is clearly of interest to know how much of this extra entry of calcium might be accounted for by membrane retrieval and how much by other routes. In comparable conditions, the extra entry of calcium is 10–30 times as great as that of mannitol, indicating that only 3–10% of the calcium entry can be due to membrane retrieval.

Our results provide strong evidence that recovery from exocytosis includes membrane retrieval by a process that involves the indiscriminate uptake of extracellular fluid. The fate of the substances taken up is unknown; but it seems possible that the process of membrane retrieval may provide a route for the uptake of physiologically important substances into the neurone.

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Food vacuole membrane in nutrient uptake by *Tetrahymena*

ALTHOUGH the ciliate protozoan, *Tetrahymena pyriformis* was first cultivated in sterile broth more than 50 years ago¹, and in chemically defined media more than 20 years ago² its uptake of nutrients in solution is poorly understood. For example, the part played by the food vacuoles is still unknown. The vacuoles seem to be essential for rapid cell multiplication in some media^{3–5}, but calculations show that the volume of the food vacuoles formed per unit time cannot account for the observed uptake of either acetate⁶ or nucleosides (present report). Previously, it was proposed that the mucous layer might be able to concentrate nutrients and thus increase their uptake⁷. I here present an alternative hypothesis.

Inclusion of insoluble material in the medium shortens generation times and induces formation of food vacuoles in *T. pyriformis*^{3–5}. The generation times are more than 40 h in particle-free, sterile-filtered proteose peptone broth, but only 6 h in this medium in the presence of particulate material (ferric or aluminium hydroxides, heat-denatured egg albumin, polystyrene beads, or the precipitate formed by heat sterilisation of the proteose peptone broth). The slowly growing cells contain less than three vacuoles per average cell, whereas the fast growing cells have 20–30 (ref. 3). These results suggest that particles induce formation of food vacuoles and that formation of food vacuoles is required for rapid uptake of one or more essential nutrients.

Data from growth experiments in media of known composi-

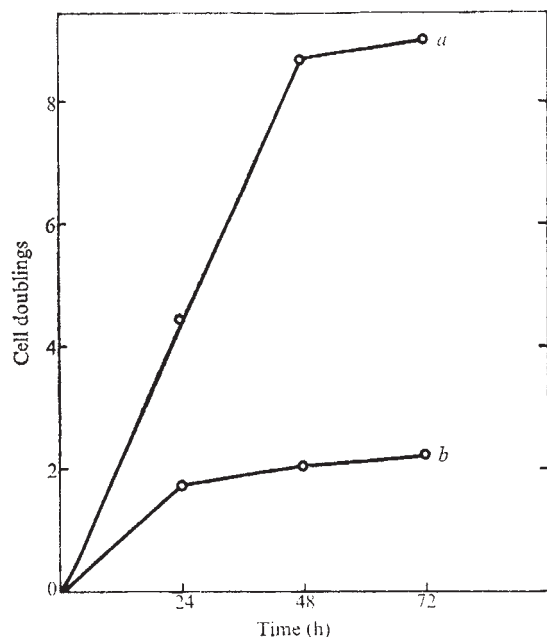


Fig. 1 Cell multiplication in populations of *Tetrahymena pyriformis* in the presence (a) and absence (b) of particulate material, Sephadex beads diameter approximately 1 μm . The nutrient medium was chemically defined except for addition of proteose peptone, final concentration 0.05%. The initial cell concentrations were 1,000 cells ml^{-1} . Each point represents the average of eight experiments. Figure reproduced from ref. 4.

tion allow the estimation of the contribution of the food vacuoles to the uptake of compounds which *Tetrahymena* cannot synthesise *de novo*, for example nucleosides⁸. Figure 1 shows cell doublings against time in the presence and absence of particles which induce the formation of food vacuoles. The estimate is based on a doubling time of 6 h in the presence of the particles, an observed rate of vacuole formation of one every 3 min, a measured vacuole diameter of 6 μm and a nucleoside concentration of 80 mg per l of medium⁴. From these values it has been calculated that in one generation time a cell of average volume about 27,000 μm^3 (ref. 9) makes vacuoles of which the total volume amounts to about 13,000 μm^3 . Assuming equal initial extracellular and intravacuolar concentrations, this volume contains about 10^{-12} g nucleosides. The cell requires, however, approximately 100×10^{-12} g nucleosides in order to double its content of nucleic acids¹⁰. Uptake of nucleosides through the cell surface is apparently insufficient to make up the difference, since cell multiplication is poor when food vacuoles are not formed due to the absence of particles (Fig. 1).

To account for the discrepancy, I propose that *Tetrahymena*, by some means other than uptake through its whole surface, can sweep nutrients from a volume of medium which is much larger than the sum of the volumes of the food vacuoles formed in the same time. It is unlikely that the stimulating effect of the particles is due to collision effects on the whole cell surface. This conclusion is based on results obtained in particle-containing media in which both cell multiplication and vacuole formation were greatly reduced in two sets of experimental conditions, namely in a mutant of *T. pyriformis*¹¹ containing no food vacuoles (unpublished experiments) and in the wild type after inhibition of vacuole formation with cytochalasin B (refs. 12, 13).

Since particles assist uptake of nutrients present in low concentrations, and neither the volumes of the vacuoles formed, nor entry through the whole cell surface can in any way account for the extent of this uptake, the major site of uptake under these conditions may well be the food vacuole membrane—especially while it is under formation and in open connection with the external medium. The oral structures maintain a strong current of fluid past the cytopharynx at the base of which the food vacuoles are formed. This current brings the membrane

of the forming food vacuole in contact with a relatively large volume of the extracellular medium and thus in contact with large amounts of nutrients. In support of this, it has been observed that food vacuoles collect Indian ink particles representing a medium volume at least 500 times as large as their own (L.R., H. E. Buhse jun., and K. Groh, in preparation).

Alternatively, nutrient uptake through the cell surface suffices for rapid multiplication in the absence of food vacuoles, when a generous supply of nutrients is maintained⁵.

The hypothesis presented here accounts for the experimental results obtained to date. Confirmation of its validity requires demonstration that food vacuole membrane is much more efficient than the rest of the cell surface in transporting such compounds as amino acids and nucleosides from dilute solution. This is possible in that reduced food vacuole formation is correlated with lengthened generation times in limiting media whether the scarcity of new vacuoles is due to lack of particles³⁻⁵, to inhibition of food vacuole formation by cytochalasin B treatment^{12,13} or to a temperature-sensitive mutation. The hypothesis reconciles the apparent conflict between vacuole volumes, nutrient concentrations and amounts of nutrients taken up. It also draws attention to a few so far neglected points in the discussion of the significance of the food vacuole in the uptake of dissolved nutrients by *Tetrahymena*.

This letter is dedicated to Professor Erik Zeuthen.

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Aerosol particles on tobacco trichomes

MARTELL¹ has described a plausible chain of events by which atmospheric radioactivity is incorporated into insoluble particles that are deposited in the lungs of tobacco smokers. The subsequent long exposure of the lungs to alpha activity is a strong candidate for the cause of bronchial cancer. Here we provide evidence for one of the steps in the sequence.

The accepted major sources of α radioactivity in the atmosphere are radon and its decay products, which condense by attaching themselves to sub-micron atmospheric particles. According to Martell, many such aerosols contact and are incorporated into the sticky tips of tobacco trichomes (spine-like protuberances on the leaves). To contact the trichome tips directly the smaller particles that carry most of the radioactivity must diffuse to the sticky, exudate-coated tips of the trichomes. Later, during tobacco curing, the sticky exudate polymerises to encapsulate the aerosols in highly insoluble particles. Thus,

when inhaled as part of tobacco smoke, the radioactive aerosols have long residence times in the deep lung.

Martell has shown that trichomes of cured tobacco are radioactive and that the most insoluble, filterable portion of tobacco smoke is the most radioactive. In support of Martell's inference that the radioactivity observed on trichomes comes from atmospheric aerosols, we present evidence that aerosols are on and inside trichome tips.

Trichomes from North Carolina flue-cured and Turkish air-cured tobacco leaves were examined in a Cwiskscan-100 equipped with a Kevex X-ray energy spectrometer. This instrument allows visual observation at high magnification and detection of elements heavier than neon in particles down to 250 Å in diameter. Silicon, sodium, chlorine, calcium and potassium were seen frequently (Fig. 1), iron and lead occasionally. Only potassium and calcium were seen regularly in all tips and stems equally. These known components of tobacco² mask attempts to observe them in sub-micron silicate aerosols.

Aerosols of > 0.5 µm diameter can be analysed individually on the surface of trichomes. These larger particles could be seen attached to most of the trichome tips of the North Carolina tobacco and half of those on Turkish tobacco. Of five individual particles analysed on North Carolina trichomes, four gave clear

signals from silicon; one was doubtful. Iron was detected in several particles. One 0.5 µm particle on Turkish tobacco contained chlorine. These compositions imply that the particles were silicate minerals and salt, respectively.

Trichome tips at positions where no exterior particles were visible showed silicon in half of the North Carolina samples. Portions other than the tips showed none. For Turkish tobacco sodium and chlorine were found on tips and stems. Silicon and lead were present in about half of the tips. Some of the silicon was associated with visible sub-micron particles.

In short, tips, like the particles on them, contain elements commonly associated with both continental and marine aerosols. The direct inference is that where individual particles are not seen, the signal comes from many tiny aerosols trapped within the exudate on the tips. This observation provides an experimental link in Martell's reasoning that radon daughter radioactivity precipitates on aerosols which diffuse to trichome tips and later become insoluble residues in tobacco smoke. Martell proved that the radon daughter radioactivity is present in trichomes; we have shown that the aerosols which normally carry this radioactivity are present in the tips.

We thank E. A. Martell and S. E. Poet for discussions and for the tobacco samples.

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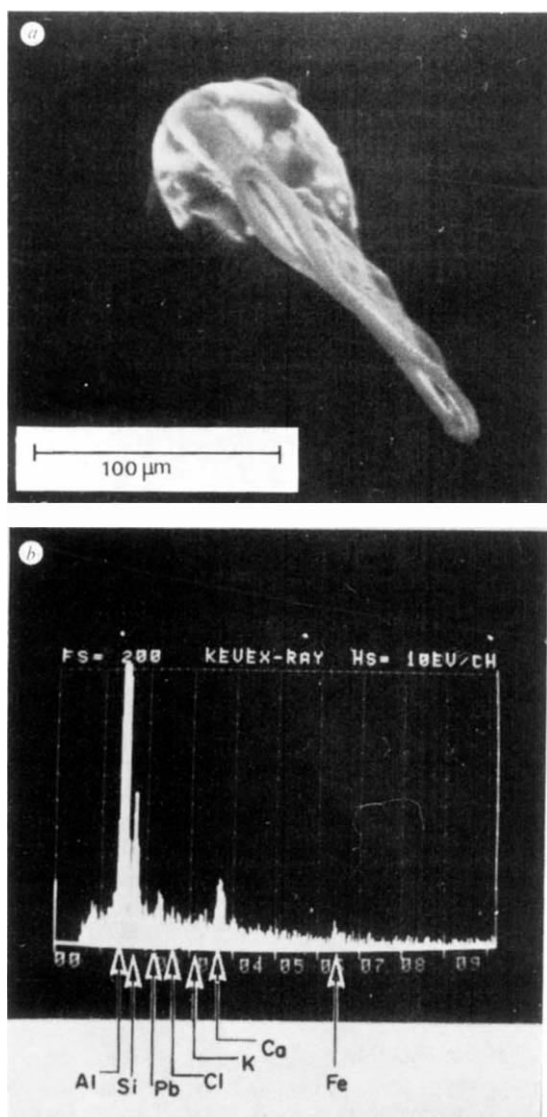
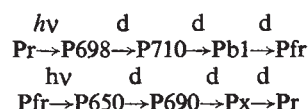


Fig. 1 *a*, Scanning electron micrograph of a cured tobacco trichome and *b*, X-ray spectrum of a trichome tip. Si, Fe, Pb, and Cl are elements commonly found in aerosols; K and Ca are components of the tobacco itself; and Al is a background signal from the sample holder.

Phytochrome intermediates in freeze-dried tissue

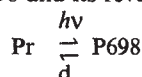
PHYTOCHROME is a plant photomorphogenetic pigment existing in two forms, Pr and Pfr, interconvertible by light, which have absorption peaks in the red and far-red regions of the spectrum respectively^{1,2}. Intermediates between Pr and Pfr have been studied by several techniques *in vivo* and *in vitro* including flash photolysis^{3,4}, low temperature spectroscopy⁵⁻⁷ and measurement of the dark (d) reactions of intermediates that accumulate in conditions of pigment cycling⁸⁻¹⁰. The pathways Pr→Pfr and Pfr→Pr have been shown to be different, but, both pathways involve an initial photoreaction of the chromophore, followed by a series of dark reactions^{10,11}:



P698, P710, P650, P690 are intermediates having peak absorbance at 698, 710, 650 and 690 nm respectively; Pb1 and Px are intermediates having relatively weak absorption bands. I now report a technique enabling phytochrome intermediates to be studied *in vivo* at 0° C using a dual wavelength spectrophotometer.

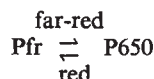
It has been known for some time that complete photo-conversion of phytochrome is not possible in dehydrated tissue or samples of phytochrome dried on to gelatin films¹²⁻¹⁵. Measurements have been made, however, either by slowly

scanning a complete spectrum or by restricting measurements to the wavelengths 730 v 800 nm used routinely for the phytochrome assay. Figure 1 shows differential absorbance changes that occur in a sample of freeze-dried dark grown *Pisum* epicotyl tissue. Using wavelengths 735 v 800 nm it is clear that Pr cannot be photoconverted to Pfr on exposure to actinic red light. Observations at 698 v 800 nm, however, demonstrate that high absorbance after actinic red light disappears in darkness. Similar measurements at the peak absorbance of Pr, 665 v 800 nm, show an increase in absorbance in darkness after actinic red light. This is interpreted as the phototransformation of Pr to the intermediate P698 and its reversion to Pr in darkness^{6,10}.



This conclusion is supported by the lack of any absorbance changes after actinic far-red light and the fact that the kinetics of the absorbance decrease at 698 nm and the absorbance increase at 665 nm are identical. It is therefore concluded that in freeze-dried tissue, Pr undergoes isomerisation of the chromophore and that this event is reversed in darkness. This situation is similar to that observed at -70°C *in vivo*¹⁰.

A similar experiment carried out with tissue freeze-dried with phytochrome in the Pfr form shows that a photoreversible reaction occurs on exposure to alternate actinic red and far-red light. A difference spectrum for this reaction is shown in Fig. 2. Compared with the normal difference spectrum of phytochrome photoconversion in hydrated tissue the absorption in the red region of the spectrum is low and is similar to that of the intermediate P650 produced on exposure of Pfr to far-red light at -196°C (refs. 6 and 10). I conclude that this photoreaction of Pfr in freeze-dried tissue is restricted to the phytochrome chromophore.



The behaviour of P650 on exposure to actinic red light is clearly different from Pr which produces the unstable P698 under these conditions of dehydration.

The effect of dehydration on phytochrome seems to be similar to that of low temperature. Low temperatures, however, not only restrict the conversion of Pr to Pfr and *vice versa*, but shift the absorption peaks of Pr and Pfr to longer wavelengths

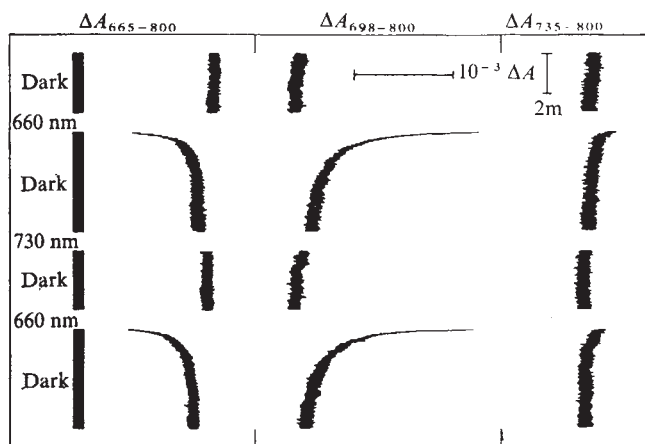


Fig. 1 Recordings of differential changes in absorbance in a sample of freeze-dried dark grown *Pisum* epicotyl tissue (3 mm path length) at 0°C using a Perkin-Elmer 156 dual wavelength spectrophotometer. They demonstrate the production of the intermediate P698 and its reversion to Pr after actinic red light. Sample consisted of 1 cm third internode sections of *Pisum sativum* cv. Alaska seedlings 7 d old grown in darkness at 25°C were frozen in liquid nitrogen, freeze-dried for 36 h and kept over P_2O_5 in a dessicator at -20°C for 5 d. All manipulations were carried out under a dim green safelight. Intensity of actinic red light (660 nm) $1.2 \times 10^3 \mu\text{W cm}^{-2}$, far-red light (730 nm) $1.3 \times 10^3 \mu\text{W cm}^{-2}$.

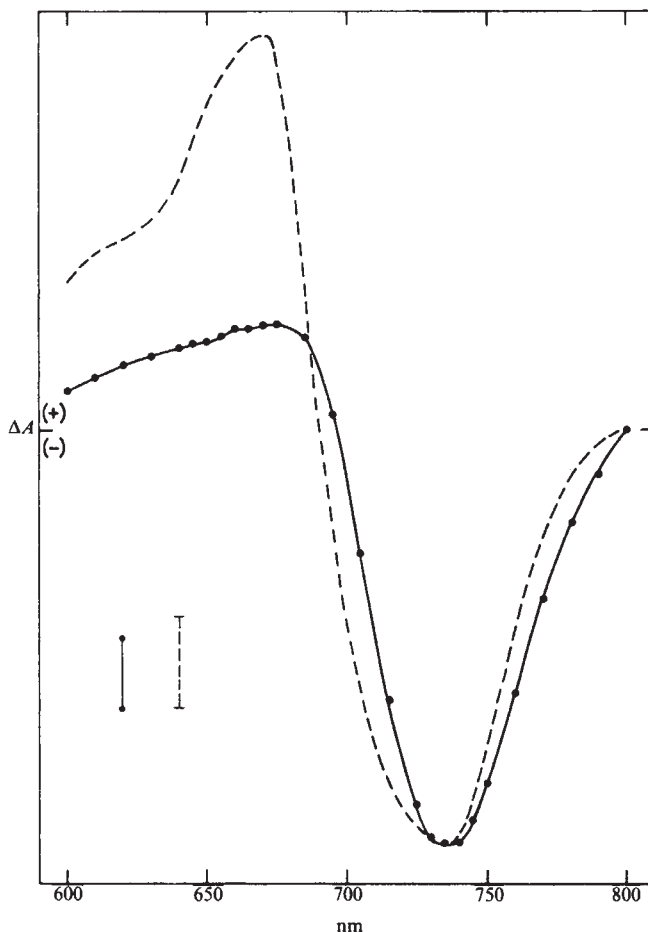


Fig. 2 —●—●, Difference spectrum for the photoreaction that occurs at 0°C in a sample of *Pisum* epicotyl tissue (3 mm path length) freeze-dried after a saturating exposure to red light (●—●, $2 \times 10^{-3} \Delta A$). It demonstrates the photoreaction between the intermediate P650 and Pfr. — — —, Difference spectrum for the photoconversion of phytochrome in a freshly collected sample of *Pisum* epicotyl tissue at 0°C (— — —, $1 \times 10^{-2} \Delta A$).

(for example, the peak absorption of Pfr shifts from 732 nm at 0°C to 744 nm at -196°C)⁶. Dehydration does not seem to bring about such large wavelength shifts. This means that the absorption characteristics of intermediates measured in this way can be used for comparison with action spectra of physiological responses in which they have been implicated. The dehydrated tissue containing Pr and Pfr shows photoreactions which are interpreted as being restricted to the chromophore of phytochrome. Controlled rehydration experiments are being conducted to study the reactions of the isomerised chromophore and protein. The use of this method should enable a critical analysis of phytochrome intermediates to be made without the difficulties associated with the use of low temperatures. Furthermore, freeze-dried samples have been demonstrated to be stable even when stored for several days in darkness at room temperature.

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Chemical and biological degradation of waste plastics

BRITISH domestic refuse can mainly be divided into ash, cinders, paper, cardboard, glass, metals, plastics and organic matter^{1,2}. The relative amounts of each component have varied over the years: ash and cinders have decreased while paper, cardboard, glass, metals and plastics have increased, because of their widespread use in 'throwaway' packaging.

Cellulosic wastes present few problems during disposal, while glass and metal can be reused or recycled. Plastics, however, are less easily dealt with. They will not rot away in the soil or during composting, and they also present problems on incineration³⁻⁵. Some plastics waste can be recycled at source within industry but at present it would be uneconomical to reuse the plastics in town waste, and even in industry there is little demand for the reuse of plastics⁶.

It is generally agreed that most of the synthetic polymers in use today resist biodegradation but that the plasticisers, used to modify the physical properties of some plastics, are biodegradable⁷⁻⁹. As most of the plastics in town waste are unplasticised⁵, they remain unchanged after all but the most destructive disposal processes, the advent of plastics has called into question the idea of 'microbial infallibility'¹⁰. If a completely new biodegradable plastics were produced it would be more expensive^{11,12} than present plastics because of the limited scale of manufacture. On the other hand, the recent production¹³ of photodegradable plastics does not take into account the fact that the majority of town waste is still used for landfilling, and is therefore not exposed to sunlight.

Town waste at present contains 1.2% by weight (5% by volume) of plastics. About 70% of these plastics are derived from packaging: they comprise (by weight of packaging plastics): 66% polyethylene, 16% polystyrene, 8% polypropylene, 4% polyvinyl chloride, 3% thermosets, and 3% miscellaneous⁵. Our aim was to develop a process for converting waste plastics to protein. We hoped to do this by chemical degradation followed by fermentation, and a literature survey suggested that oxidation¹⁴⁻¹⁶ would be an effective degradation method. Indeed, previous experiments^{9,17} had shown that the oxidation products of polyethylene could support the growth of many thermophilic fungi and actinomycetes. We tried the effect of several oxidising agents on polyethylene, the commonest packaging plastic, and as a result of our experiments chose nitric acid (reaction conditions given in Figs 1-3). We found that the nitric acid could be recovered by distillation and reused. We were able to oxidise four 10 g batches of

polyethylene with one 250 ml batch of 86% v/v nitric acid.

We then took some discarded plastics packages, identified the plastics from their behaviour on burning⁵, and refluxed each of them with 86% v/v HNO₃. The polyethylene articles were completely dissolved after 23 h reflux, while polystyrene, polypropylene and polyvinyl chloride left residues.

We identified some of the oxidation products by gas chromatography. Oxidised polyethylene consisted of a mixture of straight-chain dicarboxylic acids containing from six to 12 carbon atoms; other components were present in trace amounts. Oxidised polystyrene consisted of two major components comprising 95% of the products. Oxidised polypropylene contained 14 components, seven of which were identified as dicarboxylic fatty acids containing 3,4,5,7,8,9 and 12 carbons. Polyvinyl chloride resisted oxidation by nitric acid.

We carried out experiments to determine whether the plastics oxidation products could support microbial growth. Nineteen thermophilic fungal species were incubated, in the conditions described in Fig. 1, with K⁺ salts of oxidised polyethylene as carbon source. (Previous experiments¹⁷ had shown that better growth was obtained on the K⁺ salts than on the dicarboxylic acids.) Little growth was obtained in the mineral salts alone, but several fungi grew well in the media containing the oxidised polyethylene. Three mesophilic fungal species cultured under the same conditions (but at 27° C) produced greater cell weights than did the thermophiles. Dry weights obtained per 50 ml of culture were: 34.2 mg for *Mucor* sp., 29.0 mg for *Penicillium* sp.,

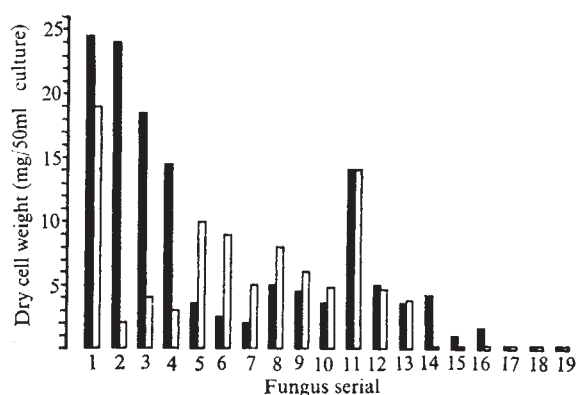


Fig. 1 Growth of thermophilic fungi on oxidised polyethylene. Polyethylene (10 g, ICI, Alkathene) was refluxed with 200 ml 60% v/v HNO₃ for 24 h. The oxidation mixture was cooled, diluted with water, then extracted into diethyl-ether. The ether extracts were bulked, washed with water, evaporated and the resulting dicarboxylic acids converted to their K⁺ salts by addition of 250 ml of 0.2 M KOH.

The K⁺ salts were incorporated at a 1.0% v/v level in 50 ml of sterile Eggins and Pugh¹⁸ mineral salts solution (without added yeast extract or L-asparagine) in 250 ml conical flasks. The medium was adjusted to pH 6.4 and inoculated with 2 mm disks of thermophilic fungi⁹ on agar. Two replicates were made at each temperature (40° C and 48° C), and after incubation, the mycelia were filtered off, washed with boiling distilled water and dried to constant weight. Controls consisted of fungi incubated with mineral salts without added oxidation products.

The figure shows the mean mycelial weight, minus the weight obtained in the controls, of the following fungi: 1, *Malbranchea pulchella* var. *sulphurea*; 2, *Cephalosporium* sp. 3, *Mucor pusillus*; 4, *Sporotrichum thermophile*; 5, *Humicola grisea* var. *thermoidea*; 6, *Humicola lanuginosa*; 7, *Mucor miehei*; 8, *Talaromyces duponti*; 9, *Talaromyces emersonii*; 10, *Thermoascus aurantiacus*; 11, *Aspergillus fumigatus*; 12, *Chaetomium thermophile* var. *coprophile*; 13, *Chaetomium thermophile* var. *dissitum*; 14, *Humicola insolens*; 15, *Sibella thermophila*; 16, *Myriococcum albobolus*; 17, *Humicola stellata*; 18, *Thielavia thermophila*; 19, *Torula thermophila*. ■, 40° C; □, 48° C.

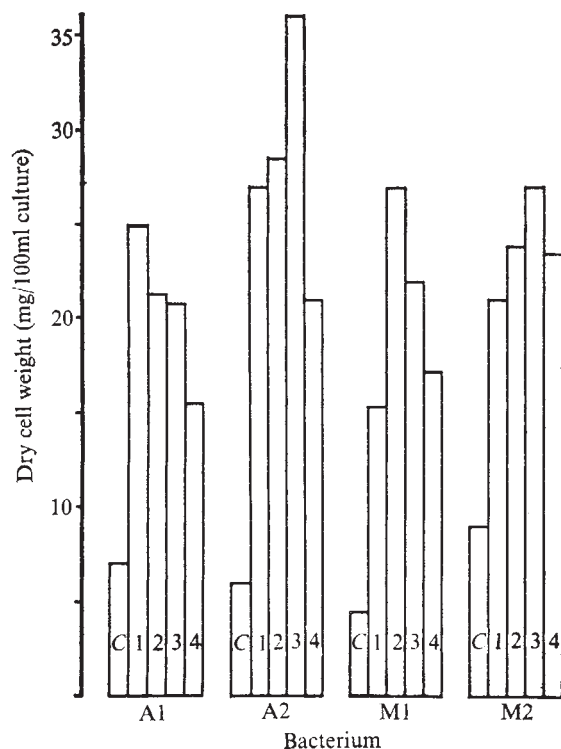


Fig. 2 Growth of mesophilic bacteria on the oxidation products of four plastics packages. Ten grams of each of the four plastics packages, 1, 'Marks and Spencer' white polyethylene bag; 2, 'Szezy' polyethylene detergent bottle; 3, 'Lancashire Hygienic Dairies' polyethylene milk bottle; and 4, 'Ovotherm' polystyrene (non-expanded) egg carton were refluxed for 24 h with 250 ml of 86% v/v HNO_3 . The oxidation mixtures were allowed to cool, then converted to their K^+ salts by adding the following volumes of 60% KOH: 1, 260 ml; 2, 260 ml; 3, 267 ml; 4, 210 ml. The K^+ salts of each oxidised plastic were incorporated at a 4.0% v/v level into a sterile nutrient salts solution after Millipore filtration. The culture medium contained: K_2HPO_4 1.0 g; KCl 0.5 g; $(\text{NH}_4)_2\text{SO}_4$ 0.5 g; MgSO_4 0.2 g; yeast extract 0.5 g; distilled water to 1.0 litre. Flasks containing 100 ml of this solution were inoculated with standard amounts of one of four bacterial cultures, A1, A2, M1, M2, and incubated at 25° C for 6 d with shaking in a Gallenkamp Orbital Incubator. Controls consisted of bacteria incubated with mineral salts without added oxidation products. After incubation, the bacteria were centrifuged off and their dry weights determined. The spent culture medium was examined for the presence of unused oxidation products (Fig. 3). C, Control; 1, polyethylene bag; 2, polyethylene detergent bottle; 3, polyethylene milk bottle; 4, polystyrene egg carton.

and 28.9 mg for *Dicoccum* sp.

Four mesophilic bacterial species (A1 and A2 isolated from the atmosphere; M1 and M2 isolated from soil) were cultured on the K^+ salts of the oxidation products of the plastics articles listed in Fig. 2. In every case the biomass exceeded that obtained in the controls, and examination of the culture medium showed that A1 and A2 removed all the polyethylene oxidation products, M1 removed 31% and M2 removed 21%. The low removal by M1 and M2 was improved by increasing the initial concentration of the oxidation products in the medium to 25% v/v; 7 d incubation then resulted in 82% and 83% removal by M1 and M2 respectively.

To obtain protein for analysis we grew 5 l batches of A1 and A2 on the K^+ salts of the oxidation products of a polyethylene milk bottle. During culture we removed a 100 ml sample each day and determined its content of oxidation products (Fig. 3) and bacterial cells: 95% of the oxidation products were removed, and the yield of bacteria reached a maximum, within 2 d. Average dry weights of 1.5 g per 5 l of each organism were obtained after 2 d growth, and the protein contents of A1 and A2 were 38.3% and 46.4% of their respective dry weights. The proteins (Table 1) contained all the essential amino acids except (for A2) methionine. They are potentially valuable as a food, although feeding trials and toxicity studies would be required to confirm this.

Higher yields of biomass were obtained from the oxidation products of polyethylene than from those of polystyrene or polypropylene. Yields (g dry weight per 100 g of plastic after 3 d growth) of A1, A2, M1 and M2 were: 43, 39, 28 and 23 respectively from polyethylene; 6, 13, 10 and 4 respectively from polystyrene; and 10, 7, 4 and 2 respectively from polypropylene. Biomass yields (g per 100 g of polyethylene) of 176, 120, 80 and 70 respectively were obtained from the first four thermophilic fungi listed in Fig. 1 after 19 d growth at 40° C. As sources of single-cell protein, fungi have three advantages over bacteria: they are easier to harvest, they have a lower nucleic acid content, and they lack a toxic lipopolysaccharide cell wall.

Although we have separated the plastics from other components of rubbish before oxidation, it is not necessary to do so. We have oxidised samples of mixed rubbish of similar composition to that of town waste, and have grown microorganisms on the oxidation products. We are not seriously proposing the oxidation of mixed town waste, but our experiments do indicate that nitric acid oxidation is effective even when the plastics have other materials mixed with them.

Table 1 Amino acid composition of protein obtained from bacteria A1 and A2 grown on polyethylene oxidation products—comparison with composition of other proteins

Protein	Essential amino acids (g/100 g protein)									Nonessential amino acids (g/100 g protein)								
	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Arg	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
Organism A1	2.1	5.6	9.5	6.7	0.4	3.6	5.5	4.0	7.7	11.7	5.6	9.3	—	10.3	9.3	4.9	4.7	1.8
Organism A2	1.9	6.3	9.1	6.5	—	3.9	5.5	4.0	8.6	12.8	4.9	9.5	—	10.5	9.5	5.5	3.5	2.0
Beef ²³	3.2	5.1	7.8	8.2	2.4	4.2	4.5	1.3	5.3	6.2	6.6	9.1	1.3	15.4	4.5	4.2	4.2	3.4
Cows milk ²³	2.7	6.2	9.9	7.8	2.4	5.1	4.6	1.4	7.0	3.7	3.7	8.2	0.8	22.2	1.9	9.8	5.8	5.6
FAO/WHO (ref. 23)	—	4.3	4.9	4.3	2.3	2.9	2.8	1.4	4.3	—	—	—	2.0	—	—	—	—	2.9

Protein was extracted from freeze-dried cells as follows^{20, 21}. Approximately 100 mg of cells were weighed into a plastic 100 ml centrifuge tube, and 40 ml of 5.0% v/v trichloroacetic acid added. After standing for 30 min at 5° C the cells were centrifuged (all centrifugations carried out at 10,000 r.p.m. for 10 min in a Martin Christ High-Speed refrigerated centrifuge, model Zeta), and the supernatant discarded. The residue (R1) was incubated for 30 min at 40–50° C with 60 ml of 75% v/v ethanol/water, centrifuged, and the supernatant discarded. The residue (R2) was incubated for 15 min at 40–50° C with a mixture of 20 ml 75% v/v ethanol/water and 20 ml diethylether, centrifuged, and the supernatant discarded. The residue (R3) was incubated for 30 min at 100° C with 40 ml of 5% v/v trichloroacetic acid, centrifuged, and the supernatant once more discarded. The resulting residue (R4) was washed twice at room temperature, first with 40 ml of ethanol which had been acidified with sufficient HCl to give a final concentration of 5 m mol l⁻¹ then with 40 ml of diethylether. Each washing was removed by centrifugation. The final residue was dried in air, weighed, and calculated as a percentage of the dry weights of bacterial cultures A1 or A2. Amino acids were analysed using a JEOL JLC-6AH Aminoacid Analyser after hydrolysing 2.0 mg of each protein in 3.0 ml of 6.0 M HCl for 18 h at 105° C, in a sealed tube. Tryptophan (destroyed by acid hydrolysis) was determined by dissolving unhydrolysed protein in sufficient 0.1 M NaOH to give an extinction of 0.2–0.5 at 290 nm, then determining the absorption at four wavelengths²².

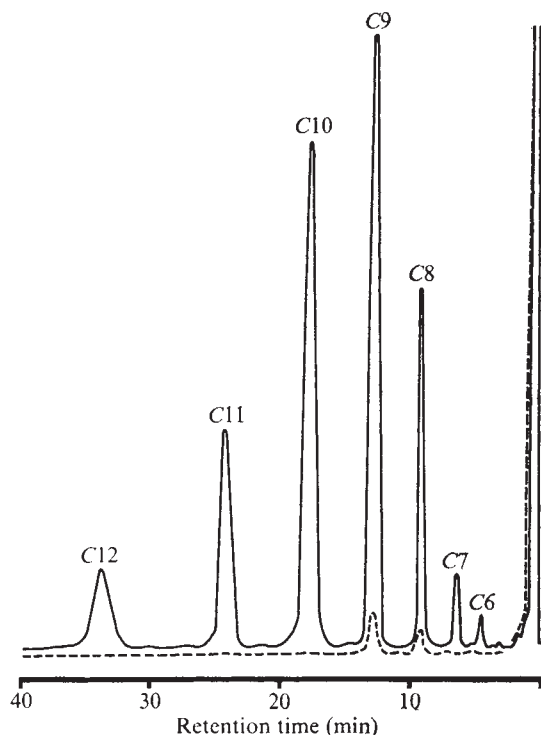


Fig. 3 Utilisation of polyethylene oxidation products by mesophilic bacterium A1. Four polyethylene milk bottles (44.5 g) were refluxed for 48 h with 890 ml 60% v/v HNO_3 , allowed to cool, and converted to their K^+ salts by addition of 60% KOH to give a final volume of 2.5 l. Two hundred millilitres of this solution were sterilised by Millipore filtration and added to 5 l of nutrient salts (Fig. 2) in a Biotec FL 110 fermenter (autoclaved at 15 lb in^{-2} for 20 min). The fermenter was inoculated with 2.0 ml of a 24 h broth culture of bacterium A1, and incubated with vigorous aeration for up to 6 d at 25° C. Each day a 100 ml sample was removed aseptically, centrifuged, and the bacterial cells dried and weighed. The supernatant was examined for the presence of unused polyethylene oxidation products as follows: it was acidified with concentrated HCl to pH 2.0, extracted with 2×100 ml diethylether, the ether extracts bulked, dried over anhydrous Na_2SO_4 , filtered, and evaporated to dryness. The dry oxidation products were converted to their dimethyl esters¹⁹ (for gas chromatography) by boiling for 3 min with 5.0 ml of 14% boron trifluoride-methanol complex (British Drug Houses). The reaction was stopped by addition of 20 ml water, and the esters extracted into 2×50 ml diethylether and evaporated to dryness. The esters were dissolved in 0.5 ml chloroform, and 1.0 μl samples injected on to the gas chromatograph. Gas chromatography was carried out on a Perkin Elmer F11 (Mark 2) dual column gas chromatograph equipped with dual flame ionisation detectors. The instrument was fitted with glass columns, 6 feet long and 3 mm internal diameter, packed with diethylene glycol succinate, 20% w/w on Chromosorb W, HMDS, 80–100 mesh. Chromatography was carried out isothermally at 165° C, with an injection port temperature of 270° C and a carrier gas (nitrogen) flow rate of 20 ml min^{-1} . Gas pressures used were: nitrogen, 40 lb in^{-2} (270 kN m^{-2}); hydrogen 20 lb in^{-2} (135 kN m^{-2}); air 30 lb in^{-2} (202 kN m^{-2}). Peak assignments were made by comparing their retention times with those of authentic dimethyl ester standards: dimethyl malonate and dimethyl sebacate (Sigma, London), dimethyl succinate, dimethyl glutarate and dimethyl suberate (Ralph N. Emanuel, Limited). —, 0 h; ---, 48 h.

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Haidinger's brushes and predominant orientation of collagen in corneal stroma

WHEN a source of bluish light, such as the sky, is viewed through polaroid glass or other polarising material, a characteristic figure known as Haidinger's brushes is fleetingly seen at the fixation point by most observers. The 'brushes' are shaped like an hourglass, yellow and darker than the surround, and are orientated with their long axis at right angles to the transmission plane of the polaroid. On either side of the brushes are light blue areas which are brighter than the surround: by some observers they are seen more clearly than the brushes themselves. If the polaroid is rotated, the brushes rotate with it in the same direction, and this manoeuvre prevents the figure from fading.

It is generally agreed that Haidinger's brushes result from the absorption of blue light by the pigment of the macula lutea^{1–3}. If the macular pigment, which absorbs maximally in the wavelength range 430–490 nm (refs 2, 4) is dichroic radially, then linearly polarised light will be absorbed about the diameter of the macula which lies at right angles to its plane of polarisation, producing a yellow region of diminished brightness with intervening blue areas occurring as a contrast effect. Nevertheless, Shurcliff⁵ argued on the basis of the appearance of the brushes with circularly polarised light that the accepted explanation of their causation might be wrong. According to Shurcliff, right-handed, circularly polarised light produces an oblique upward-to-the-right orientation of the brushes in each eye and left-

handed light produces an upward-to-the-left obliquity irrespective of the orientation of the circular polariser. Brindley³ has suggested that the effect of circularly polarised light could be explained if any of the optical media of the eye such as the cornea were birefringent with axes vertical and horizontal.

I have repeated Shurcliff's observations using quarter wave plate compensators as a source of circularly polarised light, but have been unable to confirm his findings either in my own eyes or in those of any subject whom I have so far examined. I have, however, found that a quarter wave plate for yellow light ($\lambda = 575$ nm, retardation checked by the method of de Sénarmont and with a Babinet compensator) consistently produces in persons, including myself, who are able to see Haidinger's brushes clearly, a reversal of the figure so that its long axis lies at right angles to its previous direction. This reversal is most evident when the transmission plane of the polaroid is vertical or horizontal, and the $\frac{1}{4}\lambda$ plate is interposed between the polaroid and either eye with its slow direction in the upward-and-outward diagonal. When the $\frac{1}{4}\lambda$ plate is inserted accurately in the upward-and-inward diagonal the figure disappears. A $\frac{1}{2}\lambda$ plate, on the other hand, produces reversal in the upward-and-inward diagonal and disappearance in the upward-and-outward diagonal.

These findings are explained if the collagen in the corneal stroma is predominantly orientated in the upward-and-outward diagonal—a view which agrees with earlier work^{2,6} on birefringence in the visual pathway. Collagen is positively birefringent with its optical axis lengthwise, so that its slow direction is along the length of the fibre. It is the only substance in the optical path that is sufficiently birefringent to influence the orientation of the figure significantly. Reversal will occur when the combined retardation of collagen and wave plate is near to half the wavelength of blue light, while disappearance will result from the circular or near circular polarisation occurring when the combined retardation is approximately a quarter or three quarters the wavelength of blue light.

To estimate the actual retardation produced by the corneal collagen in the upward-and-outward diagonal in my own eyes, I observed the effects of introducing into the visual path one or more compensators made from thin commercial polythene with a retardation of $\frac{1}{12}\lambda$. With the slow direction of the compensator in the upward-and-inward diagonal, that is, in the subtraction position with respect to the corneal collagen, the definition of the figure was enhanced by a retardation of $\frac{1}{12}\lambda$ and returned to normal with a retardation of $\frac{1}{6}\lambda$. These findings are compatible with a retardation due to the collagen of approximately $\frac{1}{12}\lambda$ (48 nm). A $\frac{1}{12}\lambda$ compensator in the upward-and-outward diagonal should, then, give the same combined retardation ($\frac{1}{6}\lambda$) as a $\frac{1}{4}\lambda$ compensator in the upward-and-inward diagonal, and so cause the figure to disappear.

This was found to be the case. It is now possible to explain more fully the results obtained with $\frac{1}{4}\lambda$ and $\frac{1}{2}\lambda$ plates. A $\frac{1}{4}\lambda$ plate in the addition position produces a combined retardation of $\frac{1}{3}\lambda$ which is approximately $\frac{2}{5} \times$ the wavelength of blue light giving reversal of the figure, and in the subtraction position a combined retardation of $\frac{1}{6}\lambda$, that is, $\frac{1}{5} \times$ the wavelength of blue light causing disappearance. A $\frac{1}{2}\lambda$ plate produces in the subtraction position a combined retardation ($\frac{5}{12}\lambda$) equal to $\frac{1}{2} \times$ the wavelength of blue light giving reversal, and in the addition position a combined retardation ($\frac{7}{12}\lambda$) equal to $\frac{3}{4} \times$ the wavelength of blue light causing disappearance.

A class experiment was conducted to determine the prevalence of a preferential orientation of the corneal collagen. One hundred students were asked to visualise Haidinger's brushes using polaroid and to report the effect on their orientation of a $\frac{1}{4}\lambda$ plate made from three layers of polythene. Eighty-three students were able to see the brushes, and of these 83% (69 students) obtained reversal with each eye when the slow direction of the $\frac{1}{4}\lambda$ plate was in the upward-and-outward diagonal and disappearance with the plate in the opposite diagonal. Because the subjects were not used to making such observations, these results may be regarded as confirmation of a

predominant upward-and-outward direction for the collagen bundles in the cornea. Recent authors⁷⁻⁹ have stated that the corneal collagen is randomly arranged, although Kokott's¹⁰ reconstruction of the superficial layer of the stroma shows a mainly oblique direction with an apparent preponderance in the upward-and-outward diagonal. It may be significant that the predominant orientation shown by the present study is in the line of pull of the tendons of the superior and inferior oblique muscles of the eye.

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The apparent heaviness of colours

EARLY this century, E. Bullough¹ showed that some combinations of colours, one above the other, are chosen as more 'natural' than other combinations, which tend to look top heavy. Various methods of measuring the apparent weight of colours were subsequently devised: Bullough's preference method, tests in which the weight of coloured blocks was judged either visually or directly by hand^{2,3,4}, and the 'weighing' of half-inch circles of coloured paper at either end of a simulated balance arm with an adjustable fulcrum⁵. There was general agreement that red and blue were the heaviest colours, yellow the lightest. But no statistical evaluation was used in the earlier work; and as the colours were surface-illuminated, the effect of colour was easily confounded with that of brightness. In fact, most investigators considered that brightness was probably a crucial factor. In the present study, an adaptation of Monroe's procedures, the effects of colour and brightness were investigated separately using larger transilluminated stimuli, with brightness carefully controlled. Our results show that the effect is independent of brightness. Coloured circles, equal in subjective brightness, differ considerably in apparent weight, while achromatic stimuli which differ in brightness are not consistently different in weight.

The display as seen by the subjects is shown in Fig. 1. Two circular holes, 10 cm in diameter with 30 cm between centres, were cut in a matt black screen. The holes were covered by ground glass, on to which the stimuli were back-projected by two slide projectors. Between the circles was a horizontal slit along which the subject could move a small luminous pointer to the 'balance point', by turning a knob below the board. The display was positioned vertically in front of the subject in a dark cubicle, so that his eyes were about level with the stimuli and the control knob was within easy reach. Movements of the pointer were recorded on an oscilloscope screen, unseen by the subject.

We tested the effects of colour in the absence of brightness differences, and the effects of brightness in the absence of colour differences. In order to simplify the procedure, each of the test stimuli was individually 'weighed' against a white stimulus of constant brightness. For the colour experiment five colours were used: Red, Orange, Yellow, Green, and Blue (Kodak

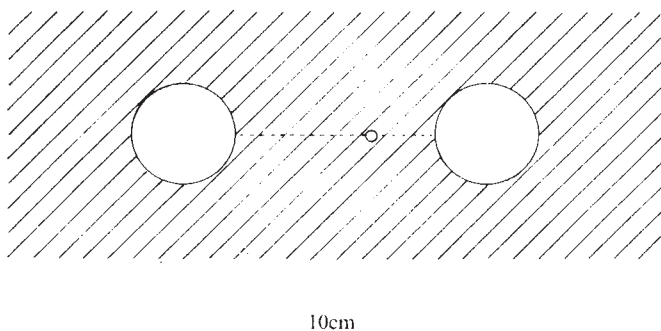


Fig. 1 The display as seen by the subjects.

Wratten filters, Nos 25, 22, 12, 58 and 38A, respectively), all adjusted to be equal in subjective brightness to the standard. For the brightness experiment white stimuli of four different brightness levels were used, covering a 25-fold range in physical intensity.

Each colour was presented eight times and each brightness level four times in the course of a testing session on one subject, giving 56 'weighings'. The brightness stimuli were mixed in amongst the colour stimuli and the whole order was randomised, with the constraint that each stimulus should appear equally often on the left and right of the display. The order was changed for different subjects to eliminate possible order effects. Medians of the judgements made to each stimulus type were used in the comparison.

Ten men and ten women took part in the experiment. All were Cambridge undergraduates, none of whom had any previous knowledge of the phenomenon being studied. Each subject was given the following printed instructions at the start of the session.

"The apparent weight of colours. Pictures are often said to have a centre of gravity, perhaps determined by the way the different colours are arranged. Early this century, those investigating the psychology of aesthetics had the idea that colours have weight. This is an experiment to test that idea.

Imagine the slit joining the two circles to be a rigid bar connecting two heavy illuminated spheres, and supported by the luminous pointer as a fulcrum. By turning the knob, move the pointer along the slit to a position about which the two spheres appear to be exactly balanced in weight. There are no right or wrong answers, so please do not feel that you need to take a long time to make these judgements."

No practice examples were given, though the subjects were encouraged to spend more time over the first few judgements so that they should get the idea. When the subject indicated, for each stimulus pair, that he was satisfied with the position of the pointer, this was recorded, and the next pair presented.

With the coloured stimuli most subjects had little difficulty in making these unusual judgements, although a few said that they did not accept the metaphor of 'weight', and were simply placing the pointer where it looked best. With the stimuli of different brightness the subjects appeared more unsure of what to do, and their judgements were rather less reliable.

To evaluate the results, the position of the pointer was expressed in terms of the displacement from the mid-point towards the test stimulus, positive displacements thus indicating

Table 2 Average ranks attributed to each colour

	Red	Blue	Green	Orange	Yellow
Men	0.70	1.65	2.25	2.10	3.30
Women	1.05	1.75	1.65	2.50	3.05
Total	0.87	1.70	1.95	2.30	3.17

0, heaviest; 4, lightest.

increasing 'heaviness' relative to the standard. The medians of the 20 subjects' median judgements for each test stimulus are given in Table 1.

All the colours were regarded as heavier than the standard, with red the heaviest, yellow the lightest and the other three clustered in between. A Friedman two-way analysis of variance by ranks indicates that the effect of colour was highly significant ($P < 0.001$). On a Wilcoxon matched pairs test, yellow comes out as significantly lighter than all the other colours ($P < 0.05$ or better), and red as significantly heavier than green, orange and yellow.

The average ranks (from 0 as the heaviest to 4 as the lightest) attributed to each colour are shown in Table 2. Though the rank ordering of the different colours was generally consistent across subjects, there was considerable variation in the absolute distance to which the pointer was displaced, some subjects tending to stick close to the mid-point, while others used nearly the full length of the slit (the inter-quartile range for red extended from 1.7–5.7 cm). Men and women gave essentially similar results.

The results for brightness showed no significant effects of any kind.

No plausible explanation has yet been offered for why people should see any equivalence between colour and weight, nor can we offer one. Bullough suggested an explanation in terms of landscape associations and aerial perspective; but he himself considered this argument *ad hoc* and unconvincing. Indirect associations, of the kind 'red = important = heavy', seem more likely. Red is commonly regarded as a particularly striking colour; moreover, in tests of colour preferences, red and blue are generally considered the most pleasant colours, yellow the least pleasant. A correlation between saliency or colour preference and apparent weight, however, if it exists, has little explanatory power. The reasons for colour preferences are themselves unclear. Whatever the explanation, the consistency with which people make such peculiar 'synaesthetic' judgements about the affective value of colours is remarkable.

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Table 1 Median displacement of pointer from mid-point towards the test stimulus

Colour	Yellow	Green	Blue	Orange	Red
	0.9	1.9	1.9	2.1	3.8 cm
Brightness*	−0.8 0.2	−0.4 0.4	+0.3 0.2	+0.7 0.3 cm	

*Brightness given in log-foot-lamberts relative to standard

Male sterility induced in barley by photoperiod

THE continuous exposure of barley plants to short photoperiods leads to marked increases in the numbers of primordia and grains per ear^{1–4}. But little is known of the effects of brief exposures to short days and here we describe the distinctive effects of such

treatments given at different stages of ear development, on the fertility of the florets of ears of barley.

Seeds of barley (cultivar 'Proctor') were sown four per 4 inch pot containing John Innes compost No. 2, in a greenhouse in March 1973. After 10 d the resultant seedlings were thinned to give a uniform stand of two plants per pot. Except when given the short day treatments, plants were grown in a 16 h photoperiod comprising 8 h of daylight (0900h–1700h) in the greenhouse and 8 h of incandescent light (1700h–0100h) of an intensity of approximately 400 lx at pot height, in adjacent photoperiod chambers. The experimental layout consisted of eight randomised blocks, with each block taking up a single trolley. The trolley positions within both the greenhouse and photoperiod chambers were randomised daily to minimise block differences.

Immediately after the appearance of double ridges on the main shoot apex, the first of four transferences of plants into short days was carried out, 21 d after sowing (treatment A). The other groups of plants were transferred 35 d (treatment B), 42 d (treatment C) and 56 d (treatment D) after sowing. These plants received a photoperiod of 10 h for 14 d, this consisting of 8 h of daylight in the greenhouse (0900h–1700h) and 2 h of incandescent light (1700h–1900h) in photoperiod chambers situated next to the ones accommodating the plants receiving long days.

During the treatment period, those plants receiving the short photoperiods were removed from the different blocks and carried on a single trolley. After the 14 d treatment period, the pots were returned to their original positions, within blocks, on the various trollies. At the beginning of each treatment, eight pots were selected at random and the plants dissected to determine the stage of development of the main shoot apex. These stages were the double ridge stage (treatment A), stamen initial stage (treatment B), awn initial stage (treatment C) and the final stage of ear differentiation with the awns longer than the spike (treatment D). The progress of ear development in the main shoot of the treated and control plants was determined by examining the apices of 16 plants sampled at weekly

intervals; the apices were fixed in 1:3 acetic alcohol and kept for subsequent examination. Sixteen pots were kept in long days throughout to serve as controls. The treated and control plants reached ripe-harvest maturity in a 2 week period at the end of July. At maturity, the numbers of ears per plant and of fertile and infertile florets for the individual ears were recorded. In the latter context the number of florets was counted from the first floret above the collar. Floret fertility is calculated as grain number divided by floret number and expressed as a percentage.

The short day treatments did not affect ear numbers per plant (Table 1). When imposed during the later stages of ear development, however, they markedly reduced the numbers of grains set per plant (Treatments C, D, Fig. 1*a*). The reductions were due to a significant depression of floret fertility rather than to any decrease in the numbers of florets produced per plant (Figs. 1*b* and *c*).

The reductions in floret fertility shown by treatments C and D were not distributed evenly between the various ears of the plant. Thus, when short days were imposed from the awn-initial stage (treatment C) the ear of the main shoot became almost wholly sterile, the ears in the axils of leaves 1 and 2 were unaffected and the ear in the axil of leaf 3 showed a small increase in fertility. In contrast, treatment D, which started when the awns of the main shoot apex were longer than the spike, caused almost total sterility of the axillary ears with the main shoot ear being hardly affected (Fig. 2).

Cytogenetic studies of main shoot ears of plants which had received treatment C showed pollen development to be abnormal. Thus 1 week before anthesis the young pollen grains were aggregated together and showed thick walls and irregularities in both size and shape; there was also a high frequency of nuclear aberrations with the occurrence of many bi- and multinucleates and micronuclei. At ear emergence, the anther contents had completely aborted and anthers were small and shrivelled. It seems likely, therefore, that male sterility was a major cause of floret sterility in the main shoot ears given treatment C.

Dissections of ovaries did not bring to light any obvious effect of treatment C on the development of the embryo sac. But this could not be regarded as conclusive proof that female development was unaffected as dissecting the ovary often led to fragmentation of the embryo sac into its constituent cells so as to make accurate comparisons difficult. Further evidence was therefore obtained from another experiment in which the florets on one side of the main shoot ears of plants which had received treatment C were cross pollinated with pollen from plants grown throughout in long days. The florets on the other side of these ears were not cross pollinated, so that any grain which set would be the result of self pollination. Grain numbers on the self pollinated side of the ear were very low, whilst those

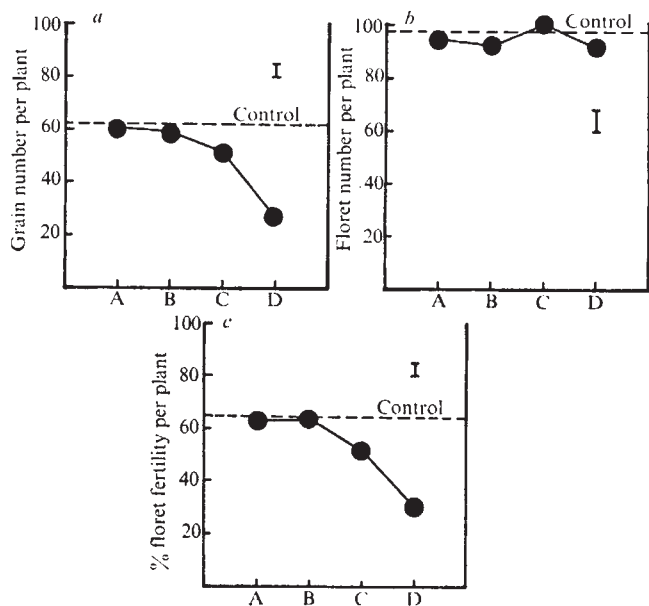


Fig. 1 The effects of a 2-week exposure to short days imposed at the double ridge stage, A; stamen-initial stage, B; awn-initial stage, C; and the later stages of awn differentiation, D on *a*, grain number per plant, *b*, floret number per plant and *c*, % floret fertility per plant. (Bar, least significant difference at $P < 0.05$.)

Table 1 Effect of photoperiod at different stages of ear development

Character	Control	A	B	C	D	L.S.D. $P < 0.05$
Ear number per plant	3.87	3.72	3.44	3.87	3.59	0.35
Total number of grains per plant	62.87	60.25	58.91	51.78	27.63	5.63
Total number of florets per plant	97.62	95.69	92.41	101.37	91.84	8.07
% fertile florets per plant	65	63	64	51	30	5
% floret fertility, main shoot	90	76	70	15	67	9
% floret fertility, leaf 1 tiller	59	67	30	53	14	16
% floret fertility, leaf 2 tiller	68	63	70	66	5	8
% floret fertility, leaf 3 tiller	55	61	77	68	6	9

A 2-week exposure to short days (10 h) was imposed at the double ridge stage, A; stamen-initial stage, B; awn-initial stage, C; and the later stages of awn differentiation, D.

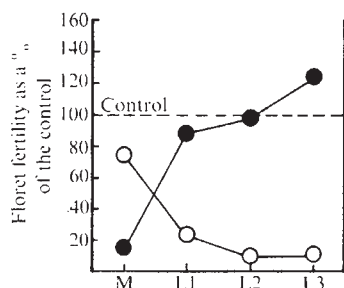


Fig. 2 The effects of a 2-week exposure to short days imposed at the awn-initial stage C ●—● and the later stages of awn differentiation D ○—○, on the fertility of the florets of main shoot ears M, and the leaf 1, 2 and 3 tiller ears (L1, L2, L3). Fertility in all ears is calculated as (Grain No.)/(Floret No.) × 100 and is plotted as a percentage of the control value.

on the cross pollinated side were considerably higher (Table 2). It seems, therefore, that the short day treatment had not affected the development of the embryo sac and that its effect on floret fertility was exerted by the induction of male sterility.

It is intriguing to consider why main shoot ears were affected by treatment C but not treatment D. It seems unlikely that the plants became unresponsive to the short day stimuli during treatment D, as the fertility of the florets of the lateral ears was markedly reduced by such treatment. It would seem more likely that pollen development is sensitive to short days only at certain stages and that in the main shoot ears these coincided with the exposure to short days in treatment C but not treatment D. The actual stages of pollen development covered by treatment C range from mitosis of the pollen mother cells at the start of treatment to the first prophase of meiosis at the end of treatment. As male and female meiosis have been shown to be synchronous in barley⁶, the embryo sac mother cell would have been at a comparable stage at the end of the treatment period.

Although any stage in the span of development covered by treatment C could be the critical process which is sensitive to short days, it may prove to be the stage of premeiotic interphase which is disrupted. This phase, in which the mitotic pollen mother cells are synchronised before entering meiosis, only occurs in male gamete formation, and it has been shown to be susceptible to changes in other environmental factors which induce varying degrees of floret sterility⁶⁻⁸.

No dissections of lateral ears were carried out but it was observed that the general development of these was later than that of main shoot ears, and it is likely, therefore, that the development of pollen in their florets lagged behind that in the main shoot ears. If this occurred, then the reduction in floret fertility in the lateral ears induced by treatment D, but not treatment C, would be consistent with the hypothesis that there is a stage during which pollen development is sensitive to short days and that this is the same for all ears.

The drastic reduction in floret fertility caused by exposure to short days is a new phenomenon. The mechanism of this effect is not clear, but phytohormones may be involved. There is evidence that levels of phytohormones in plant tissues change in

response to day length^{9,10}, and that such substances can affect floret fertility. Thus application of etrel decreases floret fertility in both barley¹¹ and wheat¹², whereas gibberellins increase fertility in male sterile lines of tomato¹³ and barley¹⁴.

The results described here imply that the use of physiologically induced male sterility may well be feasible in the controlled hybridisation of barley varieties, particularly where a rapid assessment of heterotic combinations is needed in the choosing of parents for the production of hybrid barley varieties.

We wish to thank Dr M. D. Bennett of the Plant Breeding Institute, Cambridge, for his assistance in the cytogenetic studies reported in this letter.

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Fluidisation as a feeding mechanism in beach flies

WE have seen groups of small (4-5 mm long) grey flies belonging to the species *Lipochaeta slossonae* Coquillett, 1896, standing, shaking and apparently feeding, or flying for short distances on stretches of wet sandy beach in La Jolla, California and San Felipe, Mexico. When undisturbed, they walked sideways or stood and shook their bodies, diagonally forward and downward, and backwards and upwards, at an estimated frequency of 5 s⁻¹. We guessed that they were fluidising the wet sand under their feet and thereby loosening some of the interstitial microflora which could then be sucked up as a kind of soup. This was confirmed by laboratory examination of the guts of several specimens, which contained the remains of large numbers of cells of dinoflagellates and diatoms. (The species of dinoflagellates were unfortunately unidentifiable since there were no cell wall remains, but they probably belonged to the genus *Amphidinium*, which comprises some of the commonest unarmoured interstitial dinoflagellates on this beach. The diatom species, however, could be readily identified by their silica walls. There were at least 10 genera, all typical of the marine interstitial community, including various species of *Navicula*, *Nitzschia*, *Pinnularia* and *Amphora*.)

The genus *Lipochaeta* has only one known species, *L. slossonae*: it is a poorly known fly, adults having been hitherto recorded only from Florida, Texas and from southern and central California^{1,2}. Its larvae and pupae are apparently still undescribed (B. A. Foote, personal communication). Very little is known about its biology

Table 2 Effects of cross-pollination with pollen from plants grown in long days throughout

	P: Cross-pollinated	Q: Self-pollinated	L.S.D. P<0.01
Grain No. (one side of ear)	4.89	0.86	2.28
% floret fertility (one side of ear)	41.82%	7.26%	19.24%

The recipient plants were exposed to 2 weeks of short days at the awn-initial stage. The florets on one side of the ear (P) were cross pollinated using pollen taken from plants kept entirely in long days, while those on the other side (Q) were self-pollinated. Figures are the mean of 25 ears.

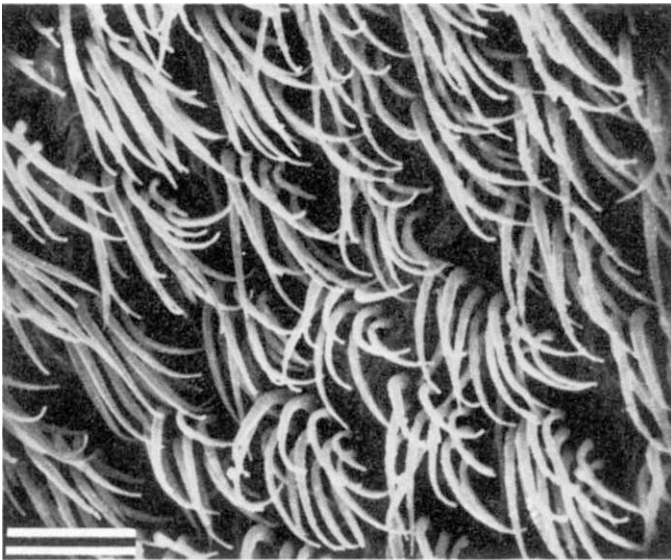


Fig. 1 Stereoscan electron micrograph taken on Cambridge S-4 SEM of portion of surface of clypeus, showing recurved hairs in bundles of 12-15. (Scale bar = 10 μm .)

apart from the fact that adults are commonly found on moist sand of ocean beaches. They are not strong fliers, but they seem to be more wary and less easily caught than the kelp flies present in the same habitat.

The head, somewhat resembling that of a fish, is flattened dorso-ventrally, with an almost flat ventral clypeus covered by small recurved hairs grouped in bundles of 12-15 (Fig. 1). This is probably an adaptation for preventing wetting of the head while the fly is feeding. The antennae, which are extremely small and lack an arista, are inserted in pits spaced far apart (Fig. 2). The mouth parts are of the normal dipteran type except for the labella, which are modified to form a filtering apparatus: it has thickened ridges approximately 8 μm apart and 10 μm long (Fig. 3), leaving pores of a size suitable for the admission of small diatoms and other algae while excluding sand grains. Further feeding adaptations of these otherwise bristle-less flies² may be the long, stout spines along their legs and the strong apical tarsal claws which enable them to stand on wet sand without wetting the velvety hair cover of their legs and body.

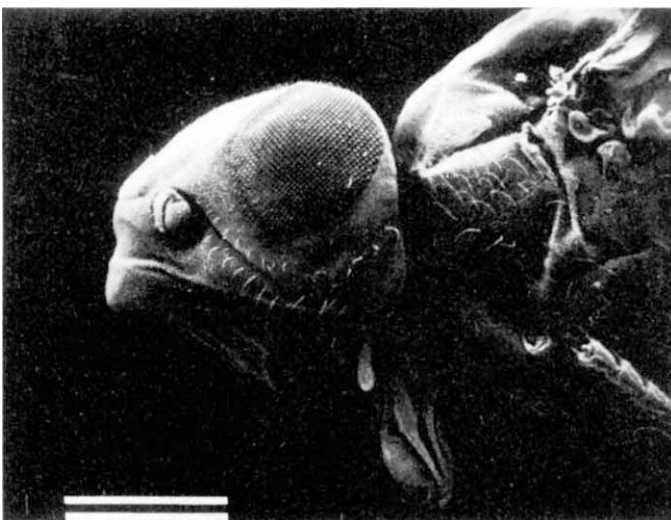


Fig. 2 Lateral view of head of *Lipochaeta slossonae*, showing flattened ventral clypeus, eye, and small antenna in pit. (Scale bar = 500 μm ; stereoscan electron micrograph taken on Cambridge S-4 SEM.)

Many adult ephydriids, like their larvae, are known to feed on microscopic algae³. Analyses of algae found in the guts of a considerable number of species are presented by Deonier⁴, who also reviewed several earlier references on the feeding habits and diets of adult ephydrid flies. A significant observation on the feeding behaviour of adult *Scatella subguttata* (Ephydriidae) is that of Brauns⁵, who refers to pressure by the anterior, boat-shaped portion of the proboscis as being somehow involved in the "licking" of food organisms from sand grains. But the phenomenon of sand fluidisation, which we observed in the feeding behaviour of *Lipochaeta*, does not seem to have been reported elsewhere. Deonier⁶ merely reported that the adults of certain species of *Hydrellia* (Ephydriidae) "exhibit peculiar behaviour while feeding, e.g., *H. biloxiae* rhythmically pushes its body up and down", without offering further explanation of this unusual behaviour. Ocypodid crabs are known to feed on interstitial microflora by rolling wet sand into pellets with their maxillipeds and then sucking out the suspension of fine organic detritus, including the microalgae⁷. Apparently some flies can exploit the same dietary niche.

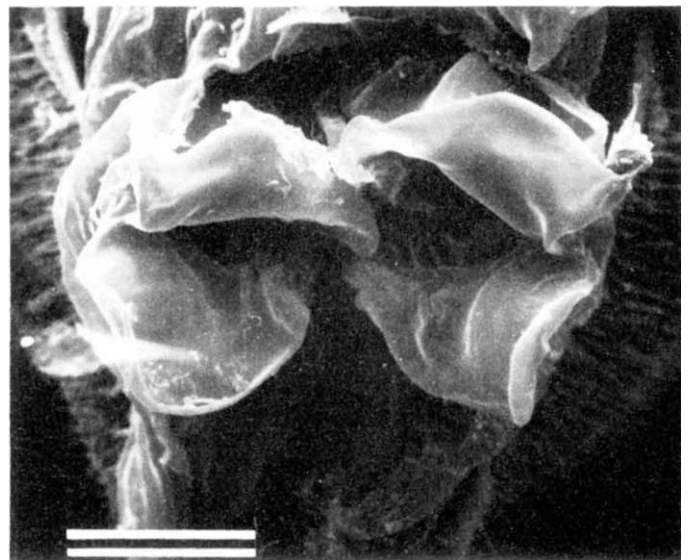


Fig. 3 Ventral view of portion of proboscis showing 'pores' on labella and small internal teeth. (Scale bar = 50 μm ; stereoscan electron micrograph taken on Cambridge S-4 SEM.)

We thank Dr W. W. Wirth, USDA, c/o US National Museum, Washington, DC, for identifying the fly species, Ellen Flentye for technical assistance with the scanning electron microscope and the Foundation for Ocean Research for financial support to L.C.

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Proof of natural selection

The Triumph of the Darwinian Method. By M. T. Ghiselin. Pp. 287. (University of California: Berkeley, Los Angeles and London, May 1973.) £1.50 paper.

IN 1909, the fiftieth anniversary of the publication of *On the Origin of Species* and the centenary of Charles Darwin's birth, two large volumes of essays on Darwin's work appeared. *Darwin and Modern Science*, edited by A. C. Seward (Cambridge University Press) and *Fifty Years of Darwinism* (Holt, New York) contained essays from many prominent scientists. Two similar volumes, *A Century of Darwin*, edited by S. A. Barnett (Heinemann, London) and *Darwin's Biological Work*, edited by P. R. Bell (Cambridge University Press) appeared for the centenary of the *Origin* in 1959. Taken together, these four volumes represented until now the most comprehensive evaluation of Darwin's scientific interests and their later influence. The problem is that each contributor to these books considered only one aspect of Darwin's thought. Quite often, contributors disagreed vigorously among themselves about Darwin's methods and significance. The result was a very disjointed appreciation of Darwin's work.

Now Michael Ghiselin has written a much needed and very different kind of book. He read Darwin carefully with the aim of elucidating the unity of conception in his major writings. Dividing Darwin's work into the themes of natural history, geology, zoology, evolution, botany, and psychology, Ghiselin convincingly demonstrates that Darwin approached each with the same general methodology. He also shows clearly how all of Darwin's diverse biological investigations, after he turned from geology, were related to his conception of evolution by natural selection. Thus the monographs on barnacles, the books on worms, climbing plants, and psychology, are seen as parts of a single conception. Ghiselin's argument, though stretched thin in some places, is important and compelling. Indeed, the book might best be titled "The Unity of Darwin's Thought".

In other ways, Ghiselin's book is less successful. His title is drawn from his answer to the question: why did Darwin attain such a remarkable success? Why did he, and not others,

write the classic works in evolutionary biology? After discounting intellect, zeal, labour, and theft from predecessors as the crucial factors, Ghiselin answers: "Darwin's success may readily be explained by a very simple hypothesis which seems not to have occurred to his critics: he thought. He reasoned systematically, imaginatively, and rigorously, and he criticized his own ideas" (page 232). In other words, the key to Darwin's accomplishments was that he "applied, rigorously and consistently, the modern, hypothetico-deductive scientific method" (page 4).

This thesis is deficient. A great number of intelligent biologists have used the same general method and have not enjoyed Darwin's success. In the final pages Ghiselin tempers his thesis by admitting that "Darwin's strength of character, his self esteem, and his ambition are in no small measure responsible for his success" (page 242); also implicated were Darwin's courage, his "remarkable talent for judging the appropriate", his ideals, and his value system. Another perhaps important reason why Darwin had greater success than his competitors is that he had the immense advantage of nearly twenty years of reflection and gathering of evidence when it came to the application of the central ideas of the *Origin* to diverse areas of biology. Even then he had no monopoly on the idea of natural selection. For example, H. W. Bates, A. R. Wallace, and Fritz Müller developed mimicry theory, which has been a mainstay of the theory of natural selection from the early 1860s to the present. Clearly the keys to Darwin's success are more complicated than Ghiselin suggests in his title and major thesis.

The picture Ghiselin paints is this: Darwin, exemplar of scientific virtue, almost singlehandedly develops the theory of evolution by natural selection and applies it to many areas of biology. Because of stupidity, belief in design or final causes, religious prejudices, and general lack of appreciation of his methods and results, Darwin's contemporaries and successors constantly misinterpret and attack the theory of natural selection. "To some extent", admits Ghiselin, "these contrary views were justified by the difficulties of the scientific problem"

(page 7). Only with R. A. Fisher's *The Genetical Theory of Natural Selection* (Oxford University Press) in 1930 is the great part of this misinterpretation cleared away, leading for the first time to a proper appreciation of Darwin's ideas. Ghiselin, himself, is finishing the mop-up of Darwin's critics begun by Fisher in 1930, and continued in his 1954 essay, "Retrospect of the Criticisms of the Theory of Natural Selection" in *Evolution as a process*, edited by Huxley, Hardy and Ford (Allen and Unwin, London). But this picture of the hiatus of proper understanding in the time between Darwin and Fisher is seriously misleading. Who can believe that all the great biologists of the late nineteenth and early twentieth centuries were too limited to understand Darwin's theories? August Weismann, Hugo de Vries, William Bateson, Wilhelm Johannsen, Jacques Loeb, Thomas Hunt Morgan, even Francis Darwin, to mention only a few: how could they have failed to appreciate Darwin's theory of natural selection when any smart college student can succeed today? Something is wrong with Ghiselin's analysis of Darwin and his critics.

Here is the problem. Ghiselin believes that the theory of natural selection is Darwin's most important contribution to knowledge. I agree. Ghiselin believes further that Darwin presented adequate verification for natural selection. I disagree, along with Darwin's successors. Noting that Darwin believed he could not see natural selection in action because of its slowness, Ghiselin refutes Darwin's critics of the nineteenth century with this argument:

"The problem is no longer with us, since the production of new species in the laboratory, by processes known to occur in nature, is a mere chore. However, direct observations on the process of speciation were by no means necessary: Darwin answered his critics by maintaining that he could verify his theory indirectly by its implication of a large number of readily ascertained facts. Actually, the attack (upon natural selection) was wholly unfounded and most unphilosophical" (page 62).

To this Ghiselin adds that "we have no 'direct' evidence for the truth of any scientific theory", implying that Darwin's critics needed no more evidence than they got, although Ghiselin himself invokes modern laboratory experiments.

The only examples of natural selection in action Darwin provided in the *Origin* were, as he clearly stated, "imaginary" (page 90 in facsimile of first edition, Harvard University Press, 1964). As even Ghiselin admits, Darwin's theory of pangenesis and his ideas about the origin and maintenance of heritable variability in natural populations were unconvincing; yet natural selection depended upon an exact understanding of this variability. There was ample room for criticism of natural selection. One need only read Thomas Hunt Morgan's 1903 critique of Darwin, *Evolution and Adaptation* (Macmillan, New York) to see why an intelligent biologist could read Darwin carefully and still reject crucial aspects of his idea of natural selection. By reading twentieth century Ghiselin has missed the sense of history Darwinism back into Darwin, torical proportion his book requires.

Despite these criticisms, this book is the best available analysis of Darwin's works, and it is sure to stimulate substantial further research. In addition, the book contains a wealth of other ideas. Ghiselin analyses aspects of scientific method, philosophy, religion, and history; he even has a comparison of human history with geology and palaeontology. His arguments are consistently bold, clear and provocative. Although readers will surely wish to challenge many of these arguments, they will find this carefully researched book a refreshing and stimulating change from the usual fare of Darwin literature.

WILLIAM B. PROVIN

Collisions in gases

Partially Ionised Gases. By M. Mitchner and Charles H. Kurger jun. Pp. 518. (Wiley Series in Plasma Physics.) (New York and London: Wiley-Interscience, November, 1973.) £12.50.

THIS book is concerned with the theory of collision-dominated plasmas and its application to practical engineering problems, particularly magnetohydrodynamic (MHD) energy conversion. It can be used as a text for graduate level courses of different emphases, by choosing appropriate parts of the nine chapters. The authors have taught the material in this way and make suggestions based on their experience in the introductory chapter.

Chapter 2 begins the book proper, introducing the physical concepts needed to understand collisional and radiative processes. More than twenty graphs for selected collision cross-sections as a function of energy are given at the end of the chapter, so that the order of magnitude of many cross sections not included can be estimated from this collection.

Chapter 3 deals in more detail with plasmas at rest and has a section on diagnostics, which unfortunately includes the erroneous statement that ion temperatures can be deduced from probe curves. The references quoted would correct this. The magnetohydrodynamic equations of motion are derived in chapter 4, after particle motion in combined static E and B fields has been discussed. This leads to a treatment of MHD power generation and other phenomena in flowing magnetised plasmas.

Collision theory as applied to elastic collisions in plasmas is described in chapter 5, where Coulomb scattering and three-body recombination appear. Radiation from plasmas is treated instructively on a semi-classical basis in chapter 6. A more formal treatment of elastic collisions on the basis of a fuller kinetic theory appears in chapter 7. The Cartesian-tensor expansion of the Boltzmann equation is introduced here and applied in chapter 8 to the calculation of transport coefficients for plasmas of all degrees of ionisation.

Inelastic collisions, which determine the degree of ionisation, are considered in chapter 9 on ionisational non-equilibrium. Departures from the Saha equation due to escape of radiation from steady-state plasmas are discussed, as are non-Maxwellian distributions and flowing plasmas.

The material is clearly presented; obviously this textbook has grown out of genuine efforts to teach. A fine selection of numerical examples is provided with each topic, and a multitude of references after each chapter. This makes the book useful as a guide to the original work. Beginners in the field of high temperature gas dynamics would be helped by the discussion of techniques for carrying out useful approximate calculations, and by the data presented in graphical and tabular form throughout the book.

The book is well printed and bound—though my copy contains some optically thin pages—and the diagrams are excellent.

P. F. LITTLE

Reproductive handbook

Female Reproductive System. Edited by R. O. Greep. Part 1: pp. 658; part 2 pp. 375. (Handbook of Physiology: A Critical Comprehensive Presentation of Physiological Knowledge and Concepts. Section 7: Endocrinology: Vol 2.) (American Physiological Society: Washington DC; Distributed by Williams and Wilkins, Baltimore, 1973.) Part 1; \$44.50; part 2; \$25.00.

This latest volume of the American Physiological Society's "Handbook of Physiology" maintains the very high standard of its predecessors. Roy Greep has obtained a galaxy of stars to write the 50 chapters on central nervous

system-pituitary-ovarian interrelationships, effects of hormones on sexual behaviour, ovary, female reproductive tract, pregnancy, immunoendocrinology, and fertility control. Greep's approach might appear somewhat parochial in that only four of the chapters have been written by authors not attached to North American laboratories, but it is a pity that his modesty prevented the addition of a chapter under his own authorship.

The various sections made very stimulating reading, though the chapters on, for example, the biosynthesis of the ovarian steroids inevitably provided heavier reading than did other chapters. In general, although each chapter overflows with pertinent information, the provision of facts has not been permitted to obliterate the fundamental concepts that are discussed. As an excellent example of this I would cite Joan Hoffmann's chapter on "The Influence of Photoperiods on Reproductive Function in Female Mammals" in which she lucidly introduces her subject and then continues, to produce a clear diagrammatic summary of the possible interplay in the rat of photoperiodic inputs, hormone levels, and neural thresholds to permit, or otherwise, the pre-ovulatory surge of luteinising hormone. Richard Michael's chapter on "The Effects of Hormones on Sexual Behavior in Female Cat and Rhesus Monkey" is equally entertaining, though it was noticeable that one quarter of the 205 references came from Michael's own publications.

The high standard of production of this volume is reflected in the excellent reproduction of all figures. This standard of illustration is particularly of note in the chapter by S. R. M. Reynolds on "Blood and Lymph Vascular Systems of the Ovary" and the chapter by Arthur Hertig and Barbara Barton on "Fine Structure of Mammalian Oocytes and Ova".

I would quibble, though, with the use of numbers in the text instead of authors' names to indicate references. References are, however, now quoted with full titles, which is an improvement since the handbook first appeared in 1959. Inevitably, reference could in general only be made to works published up to early 1971, and, as a result, some areas of current interest such as those of releasing factors or prostaglandins are not up to date nor given much coverage.

I would, however, recommend unequivocally that anyone working in the field of reproductive physiology should invest in this volume. It certainly fulfils the stated intention of providing a reference 'bible' for predoctoral study, for preliminary orientation in preparation for research, and for preparation of lectures.

BAREND TER HAAR

obituary

I. Lakatos

IMRE LAKATOS, Professor of Logic at the London School of Economics, was born in Hungary in 1922 and died suddenly in London on February 2, 1974. He was a forceful and original thinker, with an equally forceful and original personality. His chief contributions lay in the philosophy and history of mathematics and of the physical sciences. To those who found the combination of philosophy with history a strange one, Lakatos replied (paraphrasing Kant), "Philosophy of science without history of science is empty; history of science without philosophy of science is blind".

Most people think that the history of mathematics, while a legitimate object of curiosity, is not essential to the understanding or philosophical evaluation of mathematics itself. In mathematics, they say, we begin with axioms and definitions, and prove theorems from them. But Lakatos asked how the axioms and definitions came to be proposed, and how the proofs came to be discovered. Answering these questions led him into the territory of mathematical discovery or mathematical heuristics. It is a strange territory, where theorems get proved and then refuted, where the theorem and its proof get elaborated to deal with the refutation, refuted again, and once more elaborated, until in the end something like an axiomatic theory emerges. Lakatos's brilliant 'Proofs and Refutations' (*Br. J. Phil. Sci.*, 1963) is a case study of this process. Its dialogue form is no accident, for according

to Lakatos, mathematical discovery is brought about, essentially, by critical debate. The dialogue sparkles, and comes nearer than any of Lakatos's writings to capturing the unique style of the man.

Working mathematicians who, after all, inhabit these regions may find Lakatos's topography of them familiar. And yet the mathematics student is still confronted with long and complicated 'definitions', which may be the end product of centuries of trial and error, and expected to understand them without knowing their history. All he can do in this situation is memorise the definitions parrot fashion. The implications of Lakatos's work for the teaching of mathematics have yet to be explored.

Philosophers of mathematics have a different reaction to Lakatos's work. They insist that what he describes is only the murky preamble to the placing of mathematical results on a firm, rigorous and certain foundation. They see nothing in it to alter their view, held since Plato, that mathematics is the repository of absolutely certain knowledge. In another important paper, 'Infinite Regress and the Foundations of Mathematics', (*Aristotelian Soc., supplementary volume*, 1962) Lakatos examined the most recent attempts to provide secure foundations for mathematical theories (Frege, Russell and Hilbert). He showed that all these failed, but failed gloriously: their so-called 'foundations of mathematics' actually turned out to be new and exciting mathematical theories (mathematical logic, set theory, and

recursive function theory, respectively).

Much of this carries over to Lakatos's more recent work in the philosophy and history of the physical sciences. Here, of course, Lakatos was heir to the critical philosophy of science of Sir Karl Popper. (Indeed, his philosophy of mathematics is in some respects an extension of Popper's views to mathematics, an extension which Popper himself never made but later welcomed.) Lakatos developed Popper's philosophy of science in two directions. One was the emphasis upon the importance of history for the rational evaluation of scientific theories. He insisted, against the dominant view in this field, that scientific contributions cannot be appraised independently of the historical development which produced them. His second main interest was in whether a critical philosophy of science, which eschews any firm foundations for scientific knowledge, can avoid scepticism and irrationalism. His 'methodology of scientific research programmes' was an attempt to show that it can. In it, he was sharply critical of both the irrationalist tendencies he detected in recent writers (notably Thomas Kuhn) and of some features of Popper's own views.

Like all original work, Lakatos's work raised more questions than it answered. He still had much to teach us about these questions, for he died at the height of his powers. All that remains is to hope that his unpublished work, which is considerable, will appear, and that the students he inspired will continue the 'research programme' which he initiated.

Announcements

Awards

Graham Higman has been awarded the De Morgan Medal and Paul M. Cohn the Senior Berwick Prize of the London Mathematical Society (corrected announcement).

Cecil A. Hoare has been awarded the Manson Medal and Alister Voller the Chalmers Medal of the Royal Society of Tropical Medicine and Hygiene.

Appointments

David Robinson has been appointed Professor of Food Science at the University of Leeds.

R. E. Cotton, B. D. Edwards, J. A. Scott and G. K. Williamson have been awarded Special Professorships at the University of Nottingham.

Erratum

In the article "Aftershocks caused by the Novaya Zemlya explosion on October 27, 1973" by Hans Israelson, Ragnar Slunga and Ola Dahlman (*Nature*, 247, 450; 1974) the distance scale in Fig. 1 should read 500 km. See also *Nature*, 248, 712; 1974.

International meetings

September 30–October 2, 1st Annual Philadelphia Symposium on Ageing (Dr Richard C. Adelman, Fels Research

Institute, 3420 North Broad Street, Philadelphia, Pennsylvania 19140).

October 1–4, 2nd International Colloquium on the Exploitation of the Oceans (Association pour l'Organisation de Colloques Océanologiques à Bordeaux, B.P. No. 315–16, 75767 Paris, Cedex 16, France).

October 3–9, 14th International Congress of Pediatrics (Sr Secretario General, XIV Congreso Internacional de Pediatría, Casilla de Correo 3177, Buenos Aires, Argentina).

October 7–14, 1st International Colloquium on Physical and Chemical Information Transfer and Regulation of Reproduction and Ageing (Dr J. G.

Vassilleva-Popova, Secretary General, The First Colloquium on Physical and Chemical Information Transfer and Regulation of Reproduction and Ageing, Department of Biophysics, Bulgarian Academy of Sciences, 13 Sofia, Bulgaria).

October 20–26, **26th International Congress of Physiological Sciences** (Secretariat, 26th International Congress of Physiological Sciences, New Delhi 1974, Department of Physiology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110016, India).

October 20–26, **11th International Cancer Congress** (General Secretariat, Eleventh International Cancer Congress, Via G. Venezian, 1 20133 Milan, Italy).

October 21–23, **4th Conference on Chemical and Molecular Lasers** (Dr W. Q. Jeffers, McDonnell Douglas Research Laboratories, PO Box 516, St Louis, Missouri 63166).

October 21–25, **25th Annual Session American for Laboratory Animal Science** (Mr Joseph J. Garvey, Executive Secretary, AALAS, 2317 W. Jefferson Street, Suite 208, Joliet Illinois 60435).

October 23, **Recent Advances in Ultra-violet and Visible Radiation Effects on the Skin** (Dr C. Ramsay, Department of Photobiology, Homerton Grove, London E9 6BX).

October 23–26, **College Park Colloquia on Chemical Evolution: Chemical Evolution of the Giant Planets** (Dr Cyril Ponnamperna, Laboratory of Chemical Evolution, Department of Chemistry, University of Maryland, College Park, Maryland 20742).

October 31–November 2, **Communication and Control in Social Processes** (American Society for Cybernetics, Suite 530, 1130 Seventeenth Street, NW, Washington DC 20036).

Reports and Publications

Great Britain

Department of Energy, Production and Reserves of Oil and Gas in the United Kingdom—a Report to Parliament by the Secretary of State for Energy, May 1974. Pp. 27. (London: HMSO, 1974.) 32p net.

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